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**EFFECTS OF SYNCHRONIZING THE HOURLY RELEASE OF ENERGY AND  
NITROGEN IN THE RUMEN ON THE METABOLISM AND PERFORMANCE  
OF GROWING AND LACTATING SHEEP.**

**BY**

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**Thesis submitted to the Open University for the degree of Doctor of Philosophy.**

**October 1998.**

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## ABSTRACT

The combinations of rate and extent of carbohydrate and nitrogen (N) release in the rumen on microbial protein synthesis and the production and metabolism of growing and lactating sheep was studied. In trial one the rate of release of organic matter (OM) and N in the rumen for 14 feed stuffs was characterised. The chemical composition and degradation characteristics varied between ingredients and also between batches of the same feed stuff. Using this data four diets were formulated and offered to growing lambs during experiments two, three and four. The diets were predicted to supply similar levels of metabolisable nutrients to the host, but differ in their predicted degree of synchrony of OM and N release in the rumen. Diets were offered at a restricted level during trials two and three to male lambs and *ad libitum* during trial four to ewe lambs. Rations were slow energy and synchronous, slow energy and asynchronous, fast energy and synchronous and fast energy and asynchronous. It was concluded from experiment two that there was no effect of hourly rumen synchrony on N retention however there were indications that there were differences in microbial growth and whole body energy metabolism. Trial three evaluated the rations for lamb growth rate, growth efficiency and carcass characteristics. It was concluded that diets supplying a synchronous release of energy and N to the rumen increased growth rates and growth efficiency of lambs, and that this may be due to differences in energy metabolism. It was concluded after experiment four that a synchronous release of energy and N in the rumen increased feed conversion efficiency of lambs, but not as a result of increased carcass weight. The aim of experiment five was to assess rumen synchrony on milk production and feed intake of Friesland ewes. Three diets were formulated to be synchronous, intermediate and asynchronous. It was concluded that rumen synchrony may offer a method to manipulate milk quality, and there were indications of differences in energy metabolism.

Part of this thesis has been published as:

Witt, M.W., Sinclair, L.A., Wilkinson, R.G. and Buttery, P.J. 1996. The effects of synchronising the rate of energy and nitrogen supply to the rumen in diets with two rates of carbohydrate release on metabolism in male lambs. *Proc. of the European Assoc. An. Prod. Conference, Norway*.

Witt, M.W., Sinclair, L.A., Wilkinson, R.G. and Buttery, P.J. 1996. The effects of rate of release of organic matter and nitrogen in the rumen on growth and metabolism of growing ewe lambs. *Proc. of the British Soc. An. Sci.* 163.

Witt, M.W., Sinclair, L.A., Wilkinson, R.G. and Buttery, P.J. 1997. Effects of synchronising the rate of energy and nitrogen supply to the rumen in diets with two rates of carbohydrate release on growth and metabolism of male lambs. *Proc. of the British Soc. An. Sci.* 2.

Witt, M.W., Sinclair, L.A., Wilkinson, R.G. and Buttery, P.J. 1998. Effects of synchronising the hourly release of energy and nitrogen in the rumen on feed intake and production of lactating ewes. *Proc. of the British Soc. An. Sci.* 21.

## **DECLARATION**

This thesis has been composed by myself and has not been accepted in any previous application for a degree. The work, of which this is a record, has been done by myself and all sources of information have been acknowledged by means of references.

Matthew W. Witt.

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## SUMMARY

A literature review was completed investigating carbohydrate and nitrogen degradation in the rumen and factors effecting rumen digestion. The combination of rate and extent of both carbohydrate and nitrogen metabolism in the rumen was examined to assess the effects on microbial biomass synthesis and efficiency of production. The concept of a synchronous release of energy and nitrogen in the rumen was described and discussed. Although work by others has shown that the efficiency of synthesis and total production of the microbial biomass may be improved by a synchronous release of energy and nitrogen in the rumen, little work has been completed to test whether this is translated into an enhancement in whole animal performance.

In trial one, samples of maize gluten meal, rapeseed meal, sunflower seed extract, winter beans, soya bean meal, sopralin, fishmeal, malt distillers dark grains, winter wheat straw, tapioca, citrus pulp, winter barley, sugar beet pulp and silage were characterised in terms of their degradability coefficients for organic matter (OM) and nitrogen (N) using four permanently rumen cannulated sheep. The data obtained were fitted to a first order model. The chemical composition and degradation characteristics differed widely between ingredients and large differences were also observed between different batches of the same feed. Based on these *in situ* degradability data and using a computer program, diets were formulated to vary in their degree of synchronicity. These rations were used in subsequent experiments.

For experiments two, three and four, four diets were formulated and then evaluated by

feeding to 16, 24 and 32 growing lambs respectively, in a 2 X 2 factorial design. In experiments two and three rations were offered at a restricted level, fed at 09.00 and 17.00 h and in experiment four diets were offered *ad libitum*. The rations differed in their degree of synchrony but were predicted to supply similar levels of metabolisable energy and protein. The diets were slow release of energy and synchronous (SS), slow release of energy and asynchronous (SA), fast release of energy and synchronous (FS) and a fast release of energy and asynchronous (FA).

Trial two aimed to evaluate the rations in terms of their effects on nitrogen retention and microbial protein production. Whole tract OM digestibility and nitrogen retention of lambs did not differ between treatments. Synchronous rations tended to increase the daily production of microbial nitrogen although this was not significant. Microbial protein production was significantly increased by diet FS compared to diet FA ( $p < 0.05$ ). Synchronising the hourly release of energy and nitrogen in the rumen (diets SS and FS) significantly reduced peak plasma urea and  $\beta$ -hydroxy butyrate (BHB) concentrations after the morning feed ( $p < 0.05$ ). It was concluded that, although there was no effect of hourly rumen synchrony on nitrogen retention there were indications of differences in energy metabolism in male lambs offered synchronous or asynchronous rations.

Trial three aimed to evaluate the rations in terms of their effects on lamb growth rate and growth efficiency and carcass composition. Animals were dosed with Co-EDTA and Cr-mordanted fibre to measure liquid and solid outflow rates respectively and rumen fluid was extracted and analysed for volatile fatty acid concentrations and proportions. Synchronising the hourly release of energy and nitrogen in the rumen significantly

improved both growth rate and feed conversion efficiency ( $p < 0.001$ ). There was no effect of treatment on outflow of either liquid or solid from the rumen. Lambs fed the synchronous rations had significantly higher concentrations of volatile fatty acids (VFA) in rumen liquor ( $p < 0.001$ ). A synchronous release of energy and nitrogen significantly increased the kidney knob and channel fat ( $p < 0.05$ ) and tended to increase the thickness of subcutaneous fat and significantly increased the weight of the rumen ( $p < 0.05$ ). It was concluded that diets supplying a synchronous release of energy and nitrogen to the rumen increased growth rates and growth efficiency of male lambs and that this difference may be due to differences in energy metabolism.

The aim of experiment four was to evaluate the four rations in terms of growth, growth efficiency, carcass characteristics, rumen metabolism and feed intake in ewe lambs when offered *ad libitum*. Animals offered synchronous rations had significantly improved feed conversion ratios compared to those offered asynchronous rations ( $p < 0.05$ ). Animals fed the asynchronous rations had significantly higher killing out proportions and larger *Longissimus Dorsi* areas than those fed synchronous rations. No effects of synchrony were observed on mean concentrations of plasma urea however synchronising the release of energy and nitrogen significantly decreased the plasma concentration of BHB. Animals offered synchronous rations had significantly lower concentrations of VFA in rumen liquor immediately before feeding ( $p < 0.01$ ). Animals offered diet FA ate significantly more food than animals offered diet FS. It was concluded that although a synchronous hourly release of energy and nitrogen in the rumen significantly increased the feed conversion efficiency of ewe lambs this was not a result of an increase in the carcass weight.

The aim of experiment 5 was to assess the effects of hourly rumen synchrony of energy and nitrogen on milk production, rumen and body metabolism and feed intake of Friesland ewes. Three diets were formulated, based on the *in situ* data from experiment 1 to be synchronous (syn), asynchronous (asy) or intermediate (int) in their hourly release of energy and nitrogen in the rumen, but to supply similar levels of metabolisable nutrients to the host. Diets were offered *ad libitum* as total mixed rations in a replicated Latin rectangle design with 3 periods. Each period consisted of 2 weeks adaptation and 2 weeks collection. Animals fed diets increasing in the degree of hourly synchrony tended to produce more milk protein per day and had a significantly lower concentration of fat in their milk ( $p < 0.001$ ). Total dry matter intakes did not differ between treatments, however ewes offered the synchronous ration tended to eat a higher proportion of their total daily intake during the first hour after fresh feed had been offered. Animals offered asynchronous rations did not alter their pattern of intake so as to improve the synchrony of the ration. Synchronising the release of energy and nitrogen significantly reduced the concentration of plasma urea ( $p < 0.001$ ), but did not alter BHB concentrations. Rumen fluid pH and concentrations of VFA did not differ between treatments. It was concluded that rumen synchrony may offer a method to manipulate milk quality and there were indications of differences in energy metabolism between Friesland ewes offered diets differing in their degree of rumen synchronicity.

Finally, the practical implications of the results of the present work and areas for future research were discussed.

## INTRODUCTION

The ability of the ruminant animal to convert structural carbohydrates and non protein nitrogen into consumable products for humans, results in them commanding a major part of the food chain. Past studies have resulted in information that have enhanced our understanding of the digestive processes of ruminants and the rumen itself, and as a consequence, various feeding strategies have been produced and subsequently refined. However, it has been suggested that the current ration formulation packages may benefit from a more mechanistic approach and a move away from nutrient supply and demand on a daily basis (Hungate, 1988).

More detailed feed characterization techniques such as the *in sacco* method are intended to reflect the way that constituent ingredients are metabolised in the rumen. This technique has recently been used to enable diet formulation using a choice of ingredients that supply energy and protein to rumen microorganisms in a chosen hourly ratio (Sinclair *et al.* 1993). Results have shown an enhancement in microbial protein efficiency leading to the proposal that this approach to ration formulation can increase the productivity of the host, reduce environmental pollution and reduce the requirement for expensive undegradable protein sources (Sinclair *et al.* 1993; 1995). However, little work has been conducted to determine whether the enhancement in rumen metabolism from matching energy and protein supply to the rumen is translated into an improvement in whole body metabolism.

The objective of the present thesis was to test the null hypothesis that diets formulated



to vary in their rates of energy and protein degradation in the rumen have no effect on growth, carcass characteristics, feed intake and lactation in the ruminant animal. To achieve this, metabolism and growth studies were completed with ewe and ram lambs, and lactational response and feed intake was measured with ewes. These experiments were conducted with diets formulated based on *in sacco* degradability data.

## **CHAPTER 1**

### **1.0 LITERATURE REVIEW**

## **1.1 Carbohydrate metabolism in the rumen**

Typically carbohydrates make up 70-80% of the total dry matter intake of ruminant rations (Nocek and Russell, 1988). McDonald *et al.* (1988) classified carbohydrates into 2 main groups, the sugars and the non sugars. The simplest sugars are the monosaccharides which can be further sub-divided according to the number of carbons present in the molecule. Polysaccharides (glycans) are chains of monosaccharides and can be homoglycan (containing a single type of monomer) or heteroglycan (containing two or more different monomers). Sniffen *et al.* (1983) described carbohydrates of importance to ruminants as either water soluble, starches, pectins or structural.

### **1.1.1 Water soluble carbohydrates**

The functional properties of various carbohydrates vary extensively and their occurrence in nature, especially in plants, is strongly related to the functional properties (Aman and Graham, 1990). Only a few carbohydrates are present in nature as monomers and due to their water solubility and reactivity these monomeric molecules are used as intermediary and primary energy carriers in plants. Because they are water soluble they are deemed immediately available to rumen microbes for metabolism (AFRC, 1990) and Sniffen *et al.* (1992), in their description of a net carbohydrate and protein system for cattle, gave an extensive list of feed ingredients with respective rates of degradation of their component parts. The immediately soluble fractions were estimated to have rates of fermentation from 75 to 350 % per hour depending on origin (Sniffen *et al.* 1992). A principal soluble sugar utilizer is the bacterium *M. elsdenii* (Theodorou and France,

### 1.1.2 Starch

Within the microbial eco-system, the ability to hydrolyse starch is principally displayed by bacteria, large entodiniomorph protozoa and some fungi (Chesson and Forsberg 1988). Although protozoa are capable of ingestion of amylolytic bacteria with whom they are engaged in competition for substrate, their primary role would appear to be the uptake and storage of large quantities of starch, and then to ferment it at a slower rate than bacteria resulting in a reduction in the build up of lactic acid (Chesson and Forsberg 1988). This action would help to limit the incidence of sub-acute and acute acidosis associated with a decrease in ruminal pH.

The principal amylase producing bacteria are *Bacteroides amylophilus*, *Selenomonas ruminantium*, *Succinimonas amylolytic* and *Streptococcus bovis* (Chesson and Forsberg, 1988). These species have limited ability to utilize other polysaccharides but their fast growth rates ensure their survival in the ruminal eco-system (Theodorou and France, 1993). The importance of starch degradation to microbial nutrition is not reflected in knowledge of the mechanism involved with starch hydrolysis. Scanning electron microscopic evidence supports the concept that attachment and colonization of grain particles are the first step to starch digestion (McAllister *et al.*, 1990) and the extracellular enzymes excreted by the bacterium are  $\alpha$ -amylases with properties similar to the  $\alpha$ -amylases isolated from mammalian or other microbial sources (Chesson and Forsberg, 1988). As digestion progresses the starch is hydrolysed to maltose units and

then glucose which is metabolised to produce energy, presumably via glycolysis (Williams and Coleman, 1988). Entodiniomorphid protozoa possessing the highest amylase activities are *Eremoplastron bovis*, *Diploplastron affine*, *Ophryoscolex caudatus* and *Polyplastron multivesiculatum* (Williams and Coleman, 1988).

Cereal grains are the most common sources of starch for ruminants and measurements of starch digestibility from different grains, have resulted in a wide range of digestibilities for starches from similar origins (Herrena-Saldana *et al.*, 1986; Erdman *et al.*, 1987; MacRae and Armstrong, 1969). Herrena-Saldana *et al.* (1990b) compared the rumen degradability of starch of five cereal grains, corn, milo, wheat, barley and oats *in vivo*. Using the model described by Ørskov and McDonald (1979) the potentially degradable fraction (a+b) for the five starches was similar. However, there were large differences in both the immediately soluble fractions and the rate of degradation of the potentially degradable fraction (Fig 1.1).

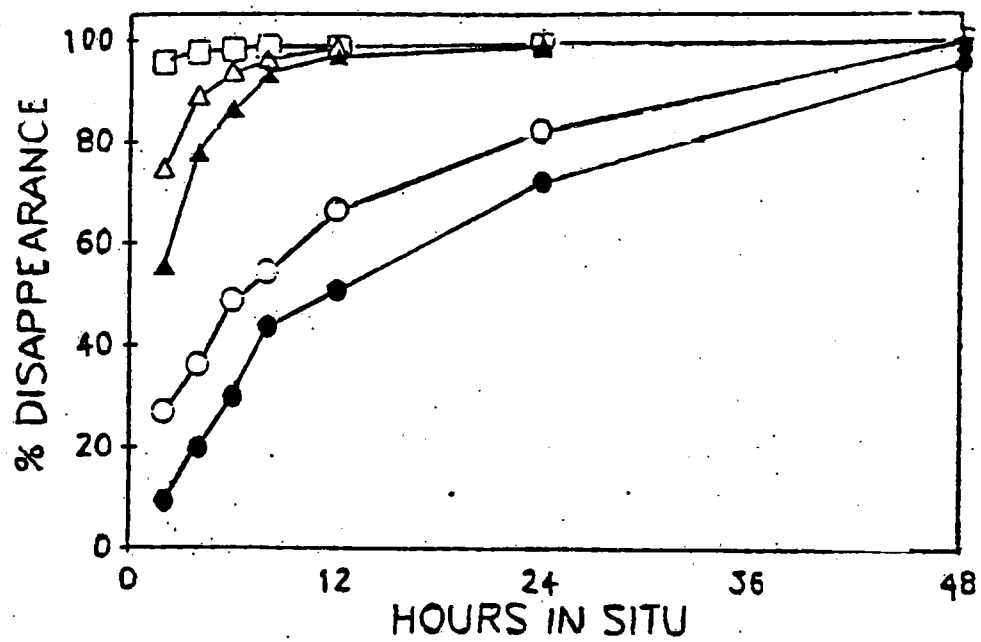


Fig. 1.1. *In situ* starch disappearance of five grains. (○ corn; ● milo; △ wheat; ▲ barley; □ oats). Herrera-Saldana *et al.* (1990b).

The differences in the rate of degradation and the immediately soluble fractions of the various starch sources will alter the amount of carbohydrate available to the microorganisms according to rumen outflow rate. Table 1.1 shows the amount of bypass starch predicted from the various starch sources when offered to a ruminant having a rumen outflow of 8 % per h, the value adopted by AFRC (1993) for a lactating ruminant. Even though the potentially degradable fraction (a+b) of the starches are similar the amount of carbohydrate available to rumen microbes ranges from 977 g/kg for oats to only 417 g/kg for milo.

Table 1.1. Degradability coefficients and levels of bypass starch of starches from different origins.

	a	b	c	undegraded starch <sup>†</sup> (g/kg)
<sup>a</sup> Barley	0.662	0.328	0.1473	125
<sup>a</sup> Corn	0.210	0.780	0.0643	442
<sup>a</sup> Milo	0.035	0.955	0.0534	583
<sup>a</sup> Oats	0.966	0.24	0.0705	23
<sup>a</sup> Wheat	0.782	0.208	0.1928	71
<sup>b</sup> Horse bean	0.425	0.575	1.01	254
<sup>b</sup> Maize	0.234	0.766	0.049	475
<sup>b</sup> Maize gluten	0.583	0.417	0.102	184
<sup>b</sup> Pea	0.732	0.286	0.163	76
<sup>b</sup> Rice bran	0.275	0.725	0.118	293
<sup>b</sup> Tapioca	0.673	0.327	0.122	130
<sup>b</sup> Wheat bran	0.832	0.168	0.236	43

<sup>†</sup>Calculated as Ørskov and McDonald (1979) assuming a rumen outflow of 0.08/h

<sup>a</sup>Adapted from Herrera-Saldana *et al.* (1990b).

<sup>b</sup>Adapted from Sauvant and Van Milgen (1995)

### **1.1.3 Factors affecting starch degradability**

The protein configuration of cereals can influence starch degradability. Starch granules can be embedded in a protein matrix in the endosperm of the cereal grain (Rooney and Pflugfelder, 1986) and this can cause the lowering of starch solubility and lead to slower rates of degradation, as associated with sorghum. Conversely the lack of a protein - starch matrix in oat endosperm facilitates rapid degradation of oat starch (Betchel and Pomeranz, 1981). Herrana-Saldana *et al.* (1990b) suggested that a potential limiting factor that can cause a lowering in the rate of wheat starch degradation is the ridged aleurone layer that surrounds the endosperm of this cultivar. The digestibility of starch can also be affected by the cellular integrity of the starch containing units, anti-nutritional factors and the physical form of the feed (Rooney and Pflugfelder, 1986).

### **1.1.4 Pectin**

Pectins consist essentially of an  $\alpha$ -1,4 linked polygalacturonic acid backbone, with rhamnose units typically linked in  $\alpha$ -1,2 bonds causing folding of the chain. They are of high molecular weight and are often referred to as "intercellular cement" (Fahey and Berger, 1988). Pectins are found in all plants but the concentration is higher in dicotyledons (Martillotti *et al.*, 1995), and relative proportions will differ considerably depending on the phylogenic origins of the plant and the part of the plant under consideration.

Although pectins are considered a structural carbohydrate (Fahey and Bergen, 1988)



pectic polysaccharides are rapidly degraded in the rumen (Gradel and Dehority, 1972). It has been observed that pectic substances from both lucerne leaf and stem were completely degraded after 12 hours incubation and that the first order rate constant was nearly 8 times that of the total plant cell wall material. Pectolytic enzymes are divided into two main groups, namely pectic esterases and pectic hydrolases (Chesson and Forsberg, 1988). Both bacteria and protozoa have been found to degrade pectin (Chesson and Forsberg, 1988) and Kopency and Hodrova (1995) have described the pectinolytic activities of the *Neocallimastix* anaerobic fungal species.

#### **1.1.5 Structural carbohydrates**

Plant cell walls (PCW) make up about 40% of the dry matter of fresh forages and 80% of the dry matter of straws (Stewart 1997, personal communication). About 90% of the PCW dry matter is composed of the major polymers cellulose, hemicellulose, pectin and lignin. Additionally up to 10% protein may be present, the major PCW protein being extension, a  $\beta$ -pleated sheet. Using light microscopy, 3 major PCW structural components have been identified. These are the middle lamella, the primary wall and the secondary wall. The primary wall is relatively loosely organised and is formed in the growing plant. The secondary wall is particularly rich in cellulose and lignin (Chesson and Forsberg, 1988).

##### **1.1.5.1 Cell wall structure**

Cellulose is composed of glucose molecules, linked with glucosidic  $\beta$ -1,4 bonds which

form chains up to 14000 units long (Martillotti *et al.*, 1995). The chains are arranged in linear fibrils possessing regions of high and low degrees of order. The highly ordered (crystalline) regions contain many hydrogen bonds within and between cellulose chains. Hemicelluloses are composed of xylose residues linked with  $\beta$ -1,4 bonds (although the xylose can be replaced by arabinose, glucuronic acid, rhamnose and/or galactose residues) and the polymer is often branched. Hemicelluloses contain two different types of polysaccharide. Cellulosans are short chain polysaccharides that form part of the cellulose fabric itself and amorphous encrusting polysaccharides which are closely associated with lignin (Fahey and Berger, 1988). Lignin is not a carbohydrate but its association with reduced cell wall degradation warrants its mention. Lignin is a complex of phenolic and acetic polymers and the negative associations connected with fibre digestibility are not fully understood (Cornu *et al.*, 1994). The chemical nature of lignins and how they are linked with the other cell wall polymers seem as important as the total amount of lignin present. Lignins first become anchored to the primary cell walls (Yamamoto *et al.*, 1989) and increases in concentration throughout the cell walls during cell wall aging (Cornu *et al.*, 1994). The degradable carbohydrates are cross linked to the phenolic monomers (p-coumaric and ferulic acid) and core lignin (Jung, 1989). Cross linking of lignin and carbohydrate by phenolic acids makes the wall more rigid and more resistant to microbial degradation (Jung, 1989). In addition to the physical barrier effect of lignin, lignin carbohydrate complexes constitute a biochemical barrier, hindering glycanases (Jung and Deetz, 1993). As degradation of non lignified surrounding carbohydrates proceeds, the soluble lignin-carbohydrate complexes are released into rumen liquor (Chesson, 1993). These phenolic acids released may have a small antimicrobial effect, which has been observed *in vitro* (Chesson *et al.*, 1982).

Cell walls are a major source of carbon to the micro flora and the complete structure can vary in digestibility within a range from c. 30-60% (Wilson, 1994). However, individual cell types that comprise cell wall material may vary in their degradability from 0-100% (Wilson, 1994). Once ingested the structural tissues of plants form large fibre matrixes and in order for them to leave the rumen via the ruminal-abomasal orifice, they must be reduced below a critical size. An essential condition for fibre degradation is the firm adhesion of bacteria to the plant cell wall (Chesson, 1993). Most cell walls consist of cellulose fibrils embedded in a matrix of other polysaccharides (hemicellulose and pectin) with varying amounts of lignification, and principle invasion routes by bacteria are through epidermal lesions and the stomata. Chesson (1993) stated that the susceptibility of cell walls to microbial attack is determined by anatomical and physical features of the substrate, which influences the ability of cells to adhere and the accessibility of cell wall polysaccharide to degradative enzymes.

Forages invariably show a substantial increase in the proportion of xylose present in residues recovered after rumen digestion, accompanied by a corresponding decrease in glucose content. Chesson (1995) explained this by suggesting that xylose is not inherently less digestible than glucose but that it simply reflects the rapid degradation of primary cell walls which have a low xylose content. The most important property of the cell wall in relation to microbial attack is the available (or accessible) surface area and the related measures of pore size and distribution (Chesson, 1995). Cell walls with low surface area ( $2-8 \text{ m}^2/\text{g}$ ) and small pore size ( $2-4 \text{ }\mu\text{m}$  radius) impede penetration of enzymes with molecular weights greater than 20 Kda. This observation has 3 major implications: (1) attack by microorganisms and their enzymes is restricted to cell

surfaces, (2) preferential degradation of a wall polymer cannot occur in the absence of diffusion of enzymes into the wall and (3) the process of degradation is determined by the cell wall surface (Chesson, 1993). The extent of degradation, which appears to be determined by the rate at which phenolic compounds (lignins) accumulate at the cell surface, depends on the chemistry of the cell wall and varies with both age and type of cell.

#### **1.1.5.2 Digestion by bacteria**

Only 3 bacterial species commonly found in the rumen, *Fibrobacter succinogenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens* are considered to be cellulolytic. Because the major matrix polysaccharides vary widely in composition and structure (see above), a single enzyme activity may cover a wide range of enzymes varying in specificity (Wong *et al.*, 1988). McDermid *et al.* (1990) found that the bacterium *Fibrobacter succinogenes* produces 6 endoglucanases, 2 cellobioxidases,  $\beta$ -glucosidases, xylanases, xyloxidases, acetylxyanoesterases, ferulic acid esterases and arabinofuranosidases.

The major cellulolytic bacteria bond closely to the surfaces of the plant cell wall material forming erosion pits as the cellulose is degraded (Cheng *et al.*, 1984). These bacteria eventually form a continuous film within which less adhesive colonies of non cellulolytic bacteria develop living off the products of primary degradation. The major route of invasion appears to be via epidermal lesions. Colonisation by entry through the stomata is only of importance for the adhesion to leaf material (Cheng *et al.*, 1984). There are

differences in the strength of adhesion exhibited by the different cellulolytic bacteria. *Ruminococcus* species only loosely associate with the cell walls, while *Bacteroides succinogenes* exhibits tight adhesion (Chesson and Forsberg, 1988). The identity of a component to mediate bacterial attachment to insoluble substrate remains unknown. Mackie and White (1990) speculated that this could be initiated by either cellulases themselves, by virtue of their ability to bind to cellulose, cellulose-binding proteins and/or the glycoprotein coat that encapsulates most cellulolytic ruminal microorganisms

The ease of digestion of cell walls can be ranked; mesophyll, phloem, immature epidermis > mature stem parenchyma, mature stem epidermis, xylem and mestome sheath (Akin, 1989; Wilson, 1993). The readily digestible cell walls are heavily colonised by bacteria and rapidly digested. In contrast, the more resistant cells are sparsely colonised (Cheng, 1984).

An effective degradation of cell walls can only be achieved by the activities of various microbial populations; hydrolytic, fermentative and methanogenic. Interspecies transfer of hydrogen to methanogens in order to keep a low partial pressure of hydrogen is particularly important to many bacterial species. It allows more energy production during fermentation through increased ATP production at substrate level phosphorylation. Such hydrogen transfer also takes place between methanogens and populations of protozoa and fungi (Durand, 1989).

It has been shown that the major cellulolytic bacteria also degrade hemicellulose, albeit to a lesser degree (Dehority, 1991). In addition some strains of the two most numerous

bacterial species in the rumen, *Prevotella ruminicola* and *Butyrivibrio fibrisolvens*, although non-cellulolytic, are capable of solubilising hemicellulose and pectin present in some forages (Dehority, 1993). In general however, cellulolytic strains solubilise hemicellulose and pectin from forages to a greater degree than do non cellulolytic strains (Morris and Van Gylswyk, 1980).

Chesson *et al.* (1986) reported that pure cultures of cellulolytic rumen bacteria were able to degrade or solubilise forage hemicelluloses regardless of their ability to utilise the solubilized oligosaccharides as an energy source. However marked increases in hemicellulose utilization were observed when hemicellulose degrading but non utilizing bacteria, were combined with a hemicellulose utilizing strain which was unable to degrade the hemicellulose from the forage (Coen and Dehority, 1970). Miron and Ben-Ghedalia (1993) have since confirmed this synergistic activity with additional bacteria strains and species as well as with different forages. Very similar synergism patterns have been observed in the digestion of forage pectin (Osborne and Dehority, 1989). In contrast to the synergistic increases noted above, some pair combinations have resulted in a decrease in hemicellulose and pectin utilization (Coen and Dehority, 1970; Miron, 1991). Fondevila and Dehority (1994) hypothesised that the bacterial interactions (negative or positive synergism) in the microbial digestion of forage structural carbohydrate might well be controlled by the order in which the individual species have access to the substrate.

### **1.1.5.3 Digestion by protozoa**

Electron microscopic techniques have confirmed that ciliate protozoa are part of the complex microbial consortia that colonize and degrade plant material in the rumen ecosystem (Amos and Akin, 1978; Bauchop, 1989). Released soluble carbohydrate from ingested plant material attract holotrich ciliates to cell wall material and the protozoa are able to adhere by means of attachment organelle. Entodiniomorphid ciliates also associate with plant material and are able to ingest small particles or engulf and adhere to larger fragments initiating primary degradation of the plant tissues (Bauchop, 1989).

Both groups of rumen ciliates produce the wide range of enzymes necessary to effect the breakdown of plant cell wall structural polysaccharides (Williams and Coleman, 1988; Williams, 1989a,b). Experiments with defaunated animals generally indicate that overall fibre digestibility is decreased (Demeyer, 1981) although not all studies have noted this result. The decrease in digestibility may arise directly as a consequence of the protozoal fibrolytic activities or indirectly due to the effects of the protozoa on rumen volume and particle retention, the size and nature of the bacterial population and the physio-chemical conditions of the ruminal environment (Williams and Withers, 1991).

The processes involved in the attachment of protozoa are poorly understood and the limited amount of literature available describing work in this area indicates the difficulties of study. Mechanisms of attachment may involve specific binding by either cell surface associated enzymes; adhesions (molecules on the microbial cell surface that bind to receptors on the plant material); or non specific ionic interaction; or a

combination of processes (Chesson and Forsberg, 1988).

#### 1.1.5.4 Digestion by fungi

The relative contribution of anaerobic fungi to the primary degradation of plant fibre has been the subject of relatively few investigations. Cultures of fungi have been shown to be able to solubilize a high proportion of the dry weight of highly lignified plant material (Joblin and Naylor, 1989) and have been shown to preferably colonize lignified tissues (Grenet and Barry, 1988). *Neocallimastix frontalis* has been seen to be able to solubilize small amounts of lignin from cell walls (Herbraund and Fèvre, 1988) due to the action of esterases which hydrolyse acetyl, uronyl or arabinosyl type bonds, existing between lignin and xylan, and release phenolic acids. This would increase the accessibility of cellulose and hemicellulose to bacteria and the other cellulolytic microorganisms. The zoospores of the fungi are attracted to fibre in the rumen by the diffusion of soluble sugars in rumen fluid by chemotaxis where they preferably colonise the stomata and damaged areas of the ingested material, and develop a thallus that penetrates the tissues by means of rhizoids (Bauchop, 1981). The mechanisms that allow penetration by the fungi are proteolytic (Wallace and Joblin, 1985) making it easier for the rhizoid to penetrate the proteinaceous layer of plant materials. *In vitro*, fungi have been shown to reduce particle size (Oprin, 1984), decrease the tensile strength of plant tissues (Akin *et al.*, 1983) and to disrupt plant physical structure (Joblin, 1989) suggesting that one of the main beneficial acts of rumen fungi is their assistance in physical breakdown of structural carbohydrates.



#### **1.1.5.5 Rates of cell wall degradation in the rumen**

The kinetics of structural carbohydrate degradation are variable (Fig 1.2) and the total potentially degradable fraction (a+b) is generally lower for forages than concentrates or other by-products. Often digestion of fibre begins after a lag phase which can be extensive for certain feed materials. For example Lindberg (1981) found the degradation of oat fibre incorporated a lag phase of 9.0 hours. The fractional degradation rate of forages would appear to be negatively correlated to the content of dry matter (Sauvant and Van Milgen, 1995) which is probably a reflection of an increased hydration time needed before microbial colonization can occur. However Susmel *et al.* (1990) reported that the disappearance of fibre was independent of its concentration in the feed and that feeds containing a greater proportion of rumen available protein (peas and beans) tended to have a faster digestion of fibre.

RESIDUE (% DM of initial sample)

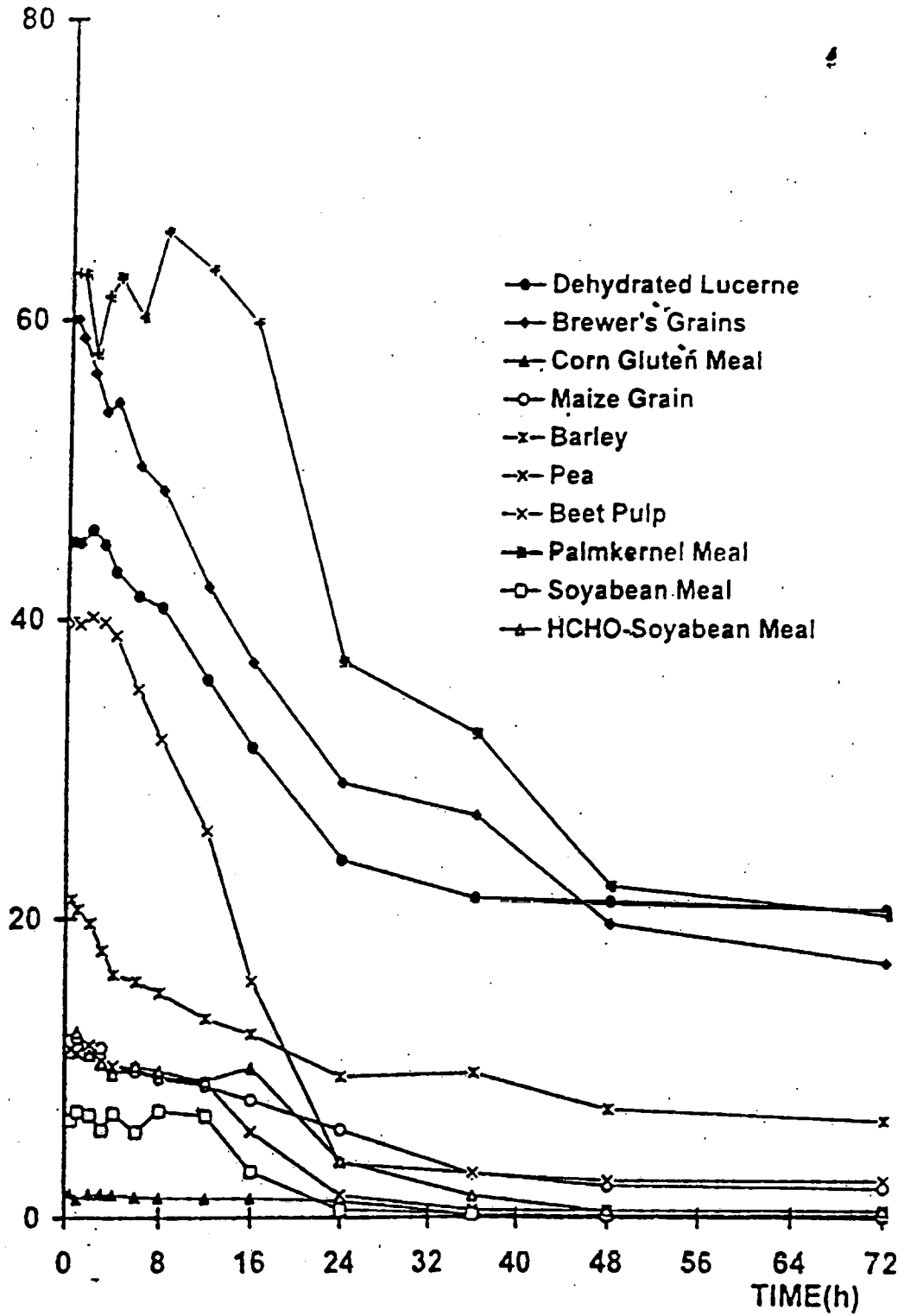


Fig 1.2. *In situ* disappearance of structural carbohydrates.  
Cited in Sauvart and Van Milgen (1995).

To increase the efficiency of use of substrates from fibre degradation attempts have been made to increase both the rate and the extent of degradability and a summary of some of these factors are presented in Table 1.2. Combinations of these factors may have additive effects; for example, chemical treatment may change the cation exchange capacity and similarly alteration of one factor may inadvertently affect another. For example grinding of forage will alter rates of hydration.

Table 1.2. A summary of some of the factors affecting the degradability of fibre.

Factor	Comments	Ref.
Rumen dilution rate	Faster dilution rates give potentially less time for digestion. Particulate turnover rate affected by particle size, density and wettability.	1
Solubility	Determined by the crystalline and macromolecular structure	2
Physical processing	Ground fibre is generally more wettable and is therefore fermented faster, has a faster exit from the rumen but can promote greater intake of absorbable substrates overall.	3,4
Chemical treatment	Exposure to alkalis causes hydrolysis of ester linkages between lignin and cell wall polysaccharides	5
Dietary lipid content	Dietary concentrations greater than 100 g/kgDM of lipid can reduce hydrolytic activities of cellulolytics	5
Cation exchange capacity	Ability of fibres to bind metal ions to cell surfaces. High cation exchange is conducive to faster rates of degradation - eg legumes and pectins	6
Readily available carbohydrate	Large amounts of readily available carbohydrate cause a drop in rumen pH suppressing cellulolytic bacteria. Small additions of readily available carbohydrate have been seen to increase fibre degradability	7,8,9,10, 11,12,13

#### References

- |                                     |                                  |
|-------------------------------------|----------------------------------|
| 1. Owens and Isaacson (1977)        | 4. Moore (1964)                  |
| 2. Huber and Herrera-Saldana (1994) | 5. McDonald <i>et al.</i> (1988) |
| 3. Beardsley (1964)                 | 6. Van Soest (1985)              |
| 7. Russell and Dombrowski (1980)    | 11. Hino and Hamano (1992)       |
| 8. Stewart (1977)                   | 12. Demeyer (1981)               |
| 9. Therion <i>et al.</i> (1982)     | 13. Muira <i>et al.</i> (1983)   |
| 10. Stewart <i>et al.</i> (1979)    |                                  |

## **1.2 Nitrogen metabolism of ruminants**

Active metabolism requires that animals must be provided with preformed essential amino acids and non essential amino acids and/or carbon skeletons accompanied by suitable nitrogen sources to synthesize non essential amino acids (Chalupa and Sniffen, 1991). In ruminants, protein requirements of the host are provided by the intestinal digestion of both microbial biomass, synthesised in the rumen and protein that is resistant to microbial attack but submits to mammalian enzymes (Mackie and White, 1990). Two of the most important influences of the pre-gastric fermentation of food by ruminants are firstly, the very effective degradation of protein and secondly, the ability of the resident microbial population to synthesize protein from non protein nitrogen sources such as urea, amines and nucleic acids (Asplund, 1986). The process of microbial protein synthesis can be so effective that Vivtanen (1966) showed that the total protein requirements of lactating dairy cows producing 10 litres could be met by protein of microbial origin alone. In order to appreciate the nitrogenous demands of rumen microbes it is important to understand rumen nitrogen metabolism. This includes types of organism present, their proteolytic mechanisms and individual needs and factors present within the feed that effect its subjectiveness to rumen fermentation.

### **1.2.1 Digestion of protein in the rumen**

The processes of protein degradation and the subsequent synthesis of rumen microbes are complex and relies on the synergistic actions of the biomass. Fig 1.3 shows important nitrogenous pools in the rumen and the movement of nitrogen between them. It can be

seen from Fig 1.3 that nitrogen can enter the rumen from a number of different sites and the path that it follows during proteolysis determines its outcome. The proteolytic activity of rumen contents varies with the nature of the diet (Wallace and Cotta, 1988) and grazing animals were found to have an activity nine times higher than animals fed dry feed (Nugent *et al.*, 1983). Protein entering the rumen has been seen to be extensively degraded by both bacterial and protozoal species (Tamminga, 1979) and anaerobic fungi (Cotta and Hespell, 1986) although the precise rate and extent of hydrolysis depends on a number of associated factors such as solubility, protein secondary and tertiary structure, cleavage of disulphide bonds and chemical and physical treatment of the feed (Nugent and Mangan, 1978).

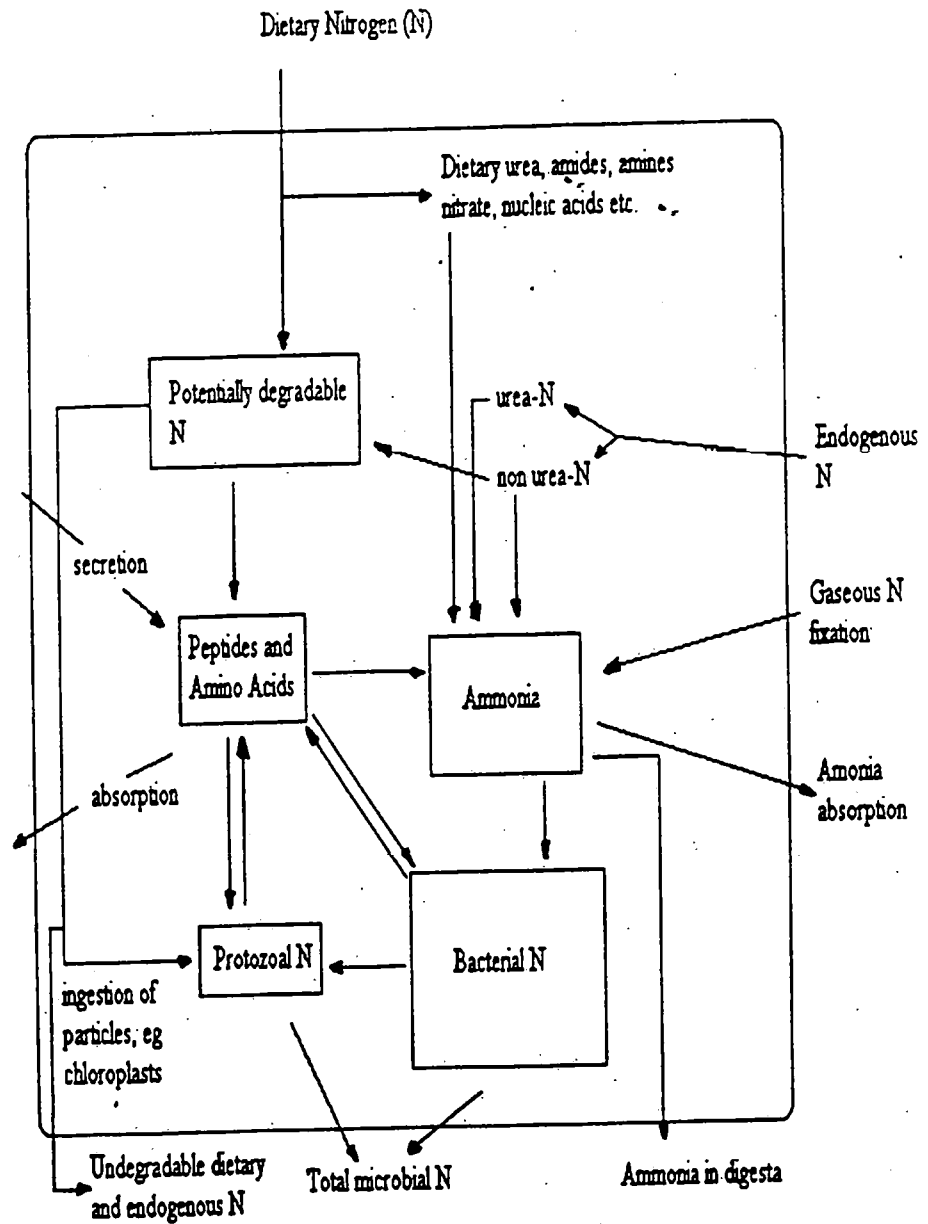


Fig. 1.3. A model of the metabolism of nitrogen in the rumen.  
(Leng and Nolan, 1984).

### 1.2.2 Proteolytic bacteria

Most species of bacteria found in the rumen have some proteolytic activity (Wallace and Cotta, 1988) with the exception of the main cellulolytic bacteria *Bacteroides succinogenes*, *Ruminococcus flavefaciens* and *R. albus*. The most active proteolytic bacterium is *Bacteriodes ruminicola* with strains of *Bacteriodes fibrisolvens*, *Clostridium spp.*, *Eubacterium ruminantium*, *Fusobacterium spp.*, *Ruminobacter amylophilus*, *Selenomonas ruminantium* and *Streptococcus bovis* also present in significant numbers depending on dietary conditions (Hazlewood *et al.*, 1983). Bacterial proteases are cell bound but are located on the cell surface to provide free access to substrate and are comprised of both exo- and endoproteases (Chalupa, 1974). The activity of bacterial proteases is predominantly of the cysteine protease type with contributions from serine- and metallo-proteases (Mackie and White, 1990). The long protein chains are absorbed onto the bacterial cell wall envelope and are hydrolysed sequentially into oligopeptides, peptides and amino acids (Wallace, 1985; Fig 1.4). Peptides of three or more amino acids are almost exclusively attacked by bacteria (Wallace *et al.*, 1990a) and results obtained by Wallace *et al.* (1990b) suggest that the main method of peptide hydrolysis is via amino- dipeptidyl peptidase activity. This possibly explains the finding that the main end products of protein degradation are dipeptides and not single amino acids (Wallace *et al.*, 1990b). Although Payne (1983) stated that the uptake of peptides by rumen microorganisms is more energetically efficient than amino acids, Wallace *et al.* (1990b) and Russell *et al.* (1983) have suggested that the uptake of dipeptides by bacteria may be the rate limiting step during protein hydrolysis indicated by an accumulation of dipeptides in rumen fluid.

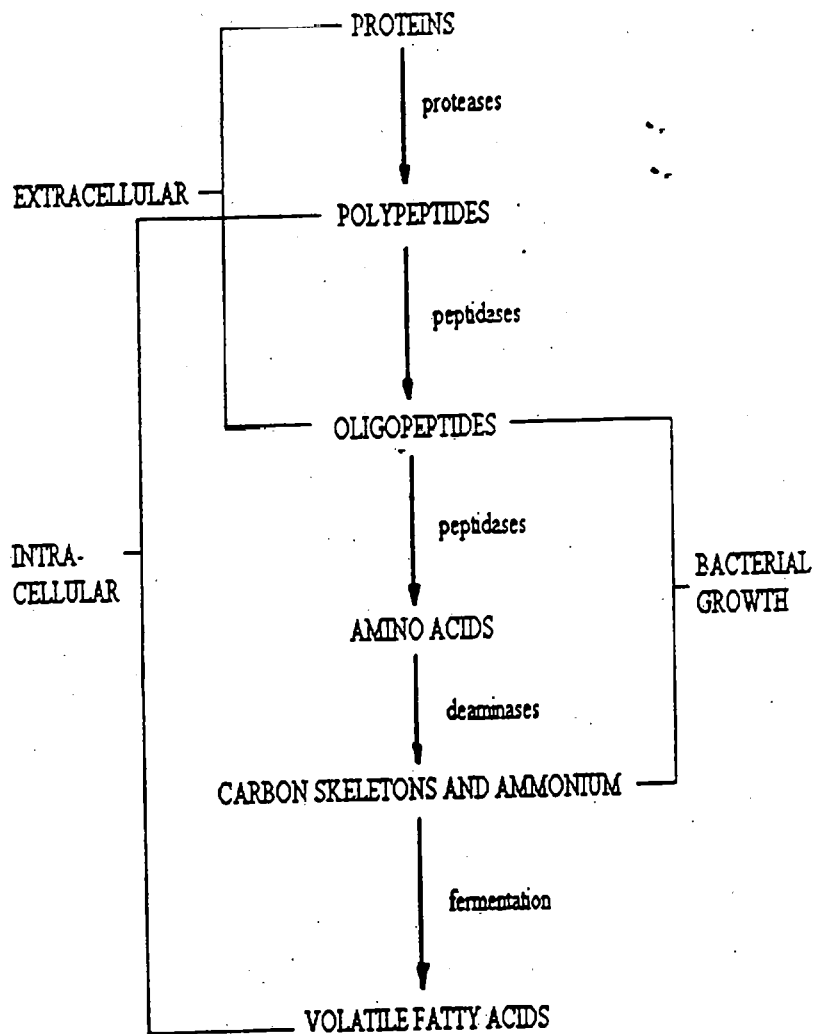


Fig. 1.4. Stepwise degradation of protein to ammonia with associated groups of enzymes (Mackie and White, 1990).



Proteolysis can not be anticipated, in terms of which protein source will be hydrolysed, since there appears to be no metabolic control over the activities of bacterial enzymes. Therefore addition of extra nitrogen as non protein nitrogen (eg urea) to a ruminant diet may not have a sparing effect on the true protein entering the rumen.

### **1.2.3 Proteolytic protozoa**

Protozoal biomass presence in rumen fluid varies greatly depending largely on the basal diet. Few protozoa occur in rumen liquor of animals fed diets containing high proportions of starch due to the low pH of the environment associated with the rapid fermentation of readily available carbohydrate (Leng and Nolan, 1984). Sources of nitrogen for growth of protozoa include ingestion of chloroplasts, engulfment of bacteria and utilization of particulate protein. The proteolytic activities of protozoa toward soluble protein is negligible compared to the actions of bacteria (Nugent and Mangan, 1981; Brock *et al.*, 1982). However protozoa have been shown to play an important role in the engulfment and subsequent hydrolysis of particulate insoluble proteins (Mackie and White, 1990). Mixed rumen protozoa exhibit a variety of protease activities, the most important of which appear to be cysteine and aspartic proteases (Wallace, 1991).

Perhaps the most significant part that protozoa play in the nitrogen metabolism of ruminants is their role in protein turn over in the rumen caused by the engulfment and hydrolysis of bacteria. This recycling of nitrogen could be expected to decrease the flow of bacterial nitrogen to the abomasum. When comparing faunated and defaunated animals Bird and Leng (1978) observed an increased food conversion efficiency in animals

without a protozoal population.

#### **1.2.4 Proteolytic anaerobic fungi**

Anaerobic fungi are recognised as a large functional group of rumen microorganisms (Bauchop, 1989) and digestion of protein is via a distinctive extracellular metalloprotease (trypsin-like) activity (Wallace, 1991). Their activity in protein hydrolysis overall is probably of limited significance with a possible exception in diets with large concentrations of forage due to the preferential colonization of fibre by fungi. Their proteolytic activity is probably of more importance in the assistance of structural carbohydrate degradation due to penetration of lignified material via the proteinaceous layers of fibrous material than it is to the nitrogen nutrition of the rumen.

#### **1.2.5 Deamination**

The end point of hydrolysis of peptides by rumen micro flora yields amino acids which are subsequently broken down more slowly than most peptides. The catabolism of amino acids by protozoa is of particular significance since their deaminative activity against amino acids is approximately three times higher than that of bacteria (Hino and Russell, 1986), produces carbon dioxide, short chain fatty acids and large quantities of ammonia. Ammonia is produced from protein by most species of protozoa, the highest activities associated with *Entodinium caudatum* and *Entodinium simplex* (Williams, 1986).

Depending on the relative constituents of the diet fermented in the rumen the rapid and

synergistic actions of bacterial, protozoal and to a lesser extent fungal proteases, peptidases and deaminases can result in 0-100 % conversion of daily nitrogen intake to ammonia. This can lead to rates of ammonia production that exceed the requirements of microbes for growth (Tamminga, 1979).

#### **1.2.6 Rates of protein degradation in the rumen**

Table 1.3 presents a list of degradability coefficients produced according to the first order model (Ørskov and McDonald, 1979) for various protein feedstuffs. The total potentially degradable fraction (a+b) is similar for all feedstuffs quoted. However differences in the proportions of the immediately soluble fraction (a), potentially degradable fraction (b) and the rate of fermentation (c) of the various feeds causes the effective degradation and hence the amount of nitrogen available to rumen microbes to be extremely variable (Table 1.3).

Table 1.3 confirms the comments of Wallace (1991), that samples of the same ingredient (soya, fishmeal, sunflower, groundnut oil cake) vary considerably between samples and experiments, making it difficult to use estimated degradability coefficients of *in sacco* data when formulating diets for ruminants.

Table 1.3. Protein degradability coefficients calculated using the nylon bag procedure of Ørskov and McDonald (1979) and effective rumen degradable protein contents calculated as Sinclair *et al.* (1993) assuming an outflow rate of 0.08 h<sup>-1</sup>

Feedstuff	Crude Protein (g/kgDM)	a	b	c	Effective RDP (g/kgCP)	ref.
Soyabean meal	na	0.224	0.776	0.095	na	1
Soyabean meal	573	0.096	0.879	0.050	434	3
Soyabean meal (full fat roasted)	400	0.210	0.790	0.032	435	4
Soyabean meal (un-heated)	349	0.28	0.72	0.091	663	4
Soyabean	350	0.528	0.457	0.310	891	5
Field peas	na	0.730	0.269	0.259	na	1
Malt distillers	223	0.660	0.280	0.086	805	2
Brewers dried grains	307	0.090	0.910	0.015	231	4
Rapeseed meal	359	0.170	0.700	0.077	513	2
Fishmeal	683	0.400	0.290	0.014	443	2
Fishmeal (Chile)	713	0.530	0.470	0.009	578	4
Fishmeal (African)	712	0.290	0.710	0.010	367	4
Fishmeal	643	0.250	na	0.010	na	5
Corn meal	94	0.235	0.373	0.055	387	3
Corn gluten feed	249	0.486	0.454	0.026	597	3
Maize gluten 20	245	0.620	0.380	0.061	784	4
Maize gluten 60	625	0.100	0.900	0.015	241	4
Maize gluten feed	190	0.686	0.252	0.140	846	5
Maize gluten meal	626	0.128	0.770	0.010	214	5
Cotton seed meal	449	0.268	0.352	0.063	423	3
Cotton seed oatcake	402	0.220	0.780	0.034	453	4
Cotton oil cake	415	0.239	0.890	0.040	536	5
Coconut oil cake	188	0.270	0.730	0.014	381	4
Ground nut oil cake	475	0.460	0.540	0.129	792	4
Ground nut oil cake	462	0.662	0.356	0.160	899	5

Table 1.3 continued.

Feedstuff	Crude Protein (g/kgDM)	a	b	c	Effective RDP (g/kgDM)	ref.
Lupin	355	0.530	0.47	0.121	813	4
Lupin	254	0.497	0.511	0.160	838	5
Poultry litter	218	0.760	0.240	0.058	861	4
Sunflower oilcake	403	0.460	0.540	0.146	809	4
Sunflower oilcake	428	0.393	0.534	0.220	785	5
Whole cotton seed	176	0.550	0.450	0.122	822	4
Whole sunflower seed	188	0.670	0.330	0.150	885	4

na = not available

## References

- 1 Aufrère *et al.* (1994)
- 2 Sinclair *et al.* (1993)
- 3 Erdman *et al.* (1986)
- 4 Erasmus *et al.* (1988)
- 5 Cronje (1983)

## 1.2.7 Factors affecting protein degradability

The process of protein digestion in the rumen is complicated and affected by a number of factors, some of which are presented in Table 1.4. The effects that these factors have on protein metabolism are not only operative as single variants but will have additional and perhaps even different effects when they act in combination.

Table 1.4. A summary of several factors affecting the rate and extent of protein hydrolysis in the rumen.

Factor	Comments	Ref.
Chemical treatment of protein	Cross linkages form with certain chemicals and amino/amide groups. Causes decrease in solubility. Chemicals include phosphonitric halides, polymerized unsaturated carboxylic acids, halotriazines, sulphonyl halides, acrolein acetals, hexamethylene-tetramine, acetylenic esters and formaldehyde.	1,
Heat	Reduction in solubility caused by Maillard reaction between $\epsilon$ -amino groups of lysine and carboxyl compounds.	2
Tannins	Tannins (polyphenolic compound) binds, via hydrogen, to protein. Tannins may reduce activity of microbial proteases	3
Physical treatment	Encapsulation of protein or amino acids with less degradable material reduces degradation	4
Modification of N-terminus of peptides	Acetylation of the $\alpha$ -amino group of peptides prevents the action of dipeptidyl amino peptidases	5
Peptide structure	Proline as the $\alpha$ -amino acid in peptides decreases degradation	6
Ammonia concentration	Reduction in number of proteolytic bacteria due to nitrogen limiting medium	7
Rate of fermentation of carbohydrate	Reduction in rumen fluid pH causes reduction in proteolytic activity - important for vegetable sources of protein.	8
Rumen outflow	At higher levels of feeding the time of exposure of protein to rumen proteolytic enzymes is reduced	8
Solubility	Proteins consisting of insoluble proteins (eg cotton seed cake and corn gluten meal contain a large proportion of prolamin and glutelin) are less degradable	9
Feeding regime	Different basal diets alter protein degradability	9
Forage maturity	Protein degradability of forage is decreased as grasses mature	10,11
Protein structure	Reversal of cross linking of di-sulphide bonds increases rate of degradation	2
Ionophores	Monensin decreases the rate of peptide and amino acid breakdown through inhibited uptake of peptides	2

#### References

- |    |                            |     |                                 |
|----|----------------------------|-----|---------------------------------|
| 1. | Chalupa (1974)             | 8.  | Ørskov (1988)                   |
| 2. | Wallace (1991)             | 9.  | Wadhwa <i>et al.</i> (1993)     |
| 3. | Broderick and Craig (1988) | 10. | van Vuuren <i>et al.</i> (1991) |
| 4. | Lynch <i>et al.</i> (1991) | 11. | Tamminga <i>et al.</i> (1991)   |
| 5. | Witt <i>et al.</i> (1997)  |     |                                 |
| 6. | Yang and Russell (1992)    |     |                                 |
| 7. | Wallace (1979)             |     |                                 |

### **1.2.8 Ammonia absorption from the rumen**

If the rate of ammonia production exceeds the rate of ammonia utilization, then large quantities can accumulate in the rumen. Under such circumstances ammonia is absorbed across the rumen wall into the blood, and is transported to the liver to undergo conversion to urea (Hogan, 1975). The fate of this urea is either to be recycled to an area of the digestive tract or excreted in urine via the kidneys. Ammonia absorption from the rumen is primarily a function of the concentration of un-ionised ammonia present in rumen fluid, itself a function of pH of the surrounding medium (Hogan, 1961).

Cocimano and Leng (1967) studied nitrogen flux in sheep fed different levels of protein, ranging from 2.8 to 35 g nitrogen per day. These differences in protein intake led to a range in plasma urea-N concentrations of from 5.6 to 72.6 mg/100 ml respectively. Their results suggested that urea entry into the rumen from blood was positively correlated to plasma concentration but, in addition, at times when ammonia absorption from the rumen was high a large fraction of urea was excreted in urine. Diets that have high contents of quickly degradable nitrogen, such as untreated grass silage, possess the potential to promote high levels of nitrogen excretion.

### **1.2.9 Evidence of the existence of an active recycling mechanism in ruminants**

Recycling of nitrogenous material occurs at three major levels in the ruminant animal. First there is the on-going process of body protein turnover in the rumen, second there are proteins and amino acids present in secretions, desquamations, abrasions and leakages

into the lumen of the gastro intestinal tract and third there are the end products of amino acid and nucleic acid catabolism which are not directly re-utilisable by the host. Urea provides the most variable contribution of quantitative significance (Egan *et al.*, 1986). The cycle in which nitrogen is transferred to the rumen from blood was originally proposed by McDonald (1948; 1952). Schmidt-Neilsen *et al.* (1957) suggested that endogenous urea is transported via saliva and by direct diffusion across the rumen epithelium into the rumen, where it can be subsequently used for amino acid synthesis by the microorganisms.

Evidence for such a systems existence is demonstrated in Fig 1.5, which depicts the efficiency of dietary nitrogen capture into microorganisms (McAllan *et al.*, 1987). The data presented have been calculated in relation to the amount of organic matter truly digested in the rumen, assuming an average nitrogen content of microbial organic matter of 100 g/kg. Diets supplying less than 16.5 g of rumen degradable nitrogen / kg OMTDR appear to support an efficiency of dietary nitrogen capture of greater than 1.0, indicating the presence of endogenous nitrogen supply to the rumen. Evidence of endogenous urea crossing the rumen epithelium from blood was also shown by Houpt (1959) who demonstrated that sheep retained 22-52 % of an intravenous dose of urea, a non protein nitrogen source. Similarly Boda *et al.* (1976) maintained sheep for 3 months on a diet free of protein, the only source of nitrogen being an intravenous infusion of urea.



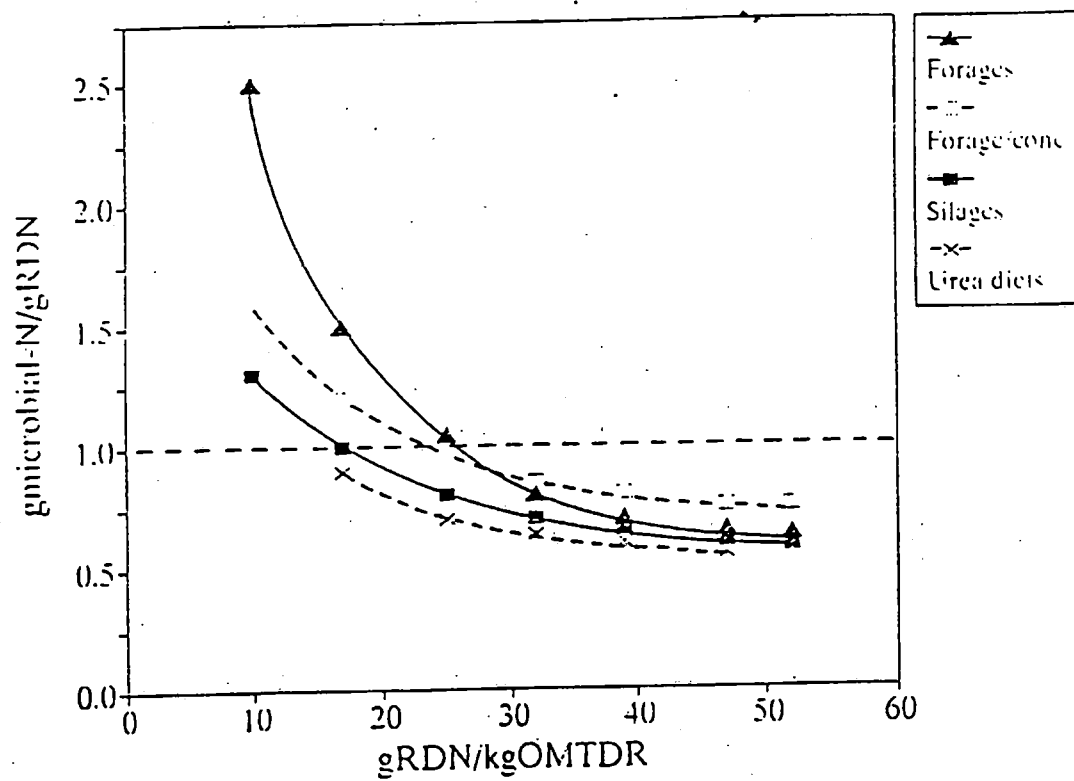


Fig 1.5. Relationship between efficiency of nitrogen capture and degradable dietary nitrogen intake.  
McAllan *et al.* (1987).

The protein regeneration cycle is probably of sufficient magnitude to sustain rumen function during times of nitrogen deprivation. However, urea is not the only source of endogenous nitrogen and endogenous proteins (enzymes, sloughed epithelial cells, digestive secretions) are also recycled to the rumen (Kennedy and Milligan, 1980). Leng and Nolan (1984) have estimated that up to 10 g (approximately 33 - 50 % of the total nitrogen intake) of endogenous nitrogen may originate from a true protein source in sheep.

#### **1.2.10 Transfer of nitrogen across the rumen epithelium**

Since the 1950's a number of research groups have investigated the passage of urea through the rumen epithelium from blood. Studies have shown arterio-venous differences of urea concentration across the rumen, and with *in vitro* preparations of rumen epithelium, revealed that urea could pass across the tissue.

A curvilinear relationship between urea concentration in the blood and rate of transfer of urea from the serosal side has been found, which has been interpreted as evidence of saturation kinetics, and concluded this to be proof of existence of an active transport system. Houpt (1970) using rinsed Pavlov pouches, originally concluded that urea flux from blood to the rumen and *vice versa* was controlled by simple diffusion. However it was noted during the experiment that saline rinsing of the pouches inhibited much of the urease activities of rumen microorganisms associated with the rumen epithelium. The experiments were repeated again, but this time making no attempt to inhibit the urease activities of the adherent micro flora and much greater amounts of nitrogen (thirteen

times the concentration of the original experiment), in the form of ammonia, appeared in the pouches. It would therefore appear that the epithelial urease is involved in some facilitatory mechanism for urea nitrogen transport into the rumen.

#### **1.2.11 Factors affecting the transfer of urea to the rumen other than rumen ammonia concentration**

A summary of other factors involved in the process of urea cycling to and from the rumen is presented in Table 1.5. Some factors will influence others, for example the extent and rate of digestion of organic matter may vary the availability of sulphur and the osmotic pressure of rumen fluid.

Table 1.5. Summary of various factors effecting the transfer of blood urea to the rumen.

Factor	Comments	Ref.
Digestion of organic matter	At a given concentration of rumen ammonia transfer of urea from blood to the rumen is positively correlated to the fermentation of organic matter. Explanations for this include - stimulatory effects of fermentation end products on microbial activity in the keratinized layers of the epithelium, increased rate of turnover of epithelial cells (specifically caused by butyrate) and/or enhancement of the number of capillaries to the rumen wall.	1,2, 3,4, 5
Growth promoters	Trenbolone acetate reduced tissue protein turnover. As a consequence urea synthesis by the liver was reduced and therefore less urea was available for recycling. In addition trenbolone acetate reduced rate of rumen fermentation which would reduce the quantity of organic matter degraded (see above).	6
Dietary sulphur	Decreasing the ratio of nitrogen:sulfur from 20:1 to 10:1 increased endogenous urea entry to the rumen via blood.	7
Osmotic pressure of rumen fluid	Increase in osmotic pressure immediately after feeding may increase the amount of endogenous urea entering the rumen via solvent drag.	8

#### References

- |   |                             |   |                           |
|---|-----------------------------|---|---------------------------|
| 1 | McIntyre (1971)             | 5 | Egan <i>et al.</i> (1986) |
| 2 | Nolan and Stachiw (1979)    | 6 | Hunter and Magner (1990)  |
| 3 | Norton <i>et al.</i> (1982) | 7 | Dhiman and Araora (1990)  |
| 4 | Kennedy (1980)              | 8 | Haupt (1970)              |

### 1.2.12 Energetic implications of nitrogen recycling in ruminants

#### 1.2.12.1 Transfer of urea across the rumen epithelium

The transfer of urea from blood to the rumen displays evidence of saturation kinetics and Cheng and Wallace (1979) concluded that urea could move into the rumen against a concentration gradient. These two findings suggest the possible presence of an active transport system for the removal of urea from blood into the rumen. The carriers of an active transport system require coupling with a source of energy, usually ATP (Blackstock, 1989). McBride and Kelly (1990) state that Na<sup>+</sup>, K<sup>+</sup> ATPase action accounts

for a sizeable proportion of oxygen consumption by the ruminal epithelial cells, and that oxygen consumption may be related to the postprandial increase in blood flow to the rumen. Although there seems to be very little data available on the specific mechanism of urea transfer from blood to rumen, perhaps the additional epithelial load of urea recycling increases oxygen demand and hence energy usage of epithelial cells because of the additional ATP needed for active transport of urea.

Houpt (1970) found that the facultative adherent rumen bacterial ureases are involved in some facilitatory mechanism for urea nitrogen transport into the rumen. Nearly all known enzymes are proteins (Stryer, 1995) and protein synthesis is dependent on high energy phosphate (ATP) utilisation for amino acid incorporation into the polypeptide chain (Milligan and Summers, 1986). The cost of enzyme synthesis is 5 ATP/ monomer synthesised (Hespell and Bryant, 1979) and because enzymes tend to be large molecules the energy requirement for their synthesis could be high. At times favourable to the transfer of urea from blood to the rumen the rate of synthesis of urease by urolytic adherent bacterial populations is likely to be high.

#### **1.2.12.2 Urea synthesis in the liver**

A major demand for ATP in the liver is for the detoxification of ammonia and the production of urea. The cost of ammonia detoxification in the ruminant animals has been estimated to be between 8 and 12 % of the total hepatic ATP use (McBride and Kelly, 1990). Lobley *et al.* (1995) infused low (25  $\mu\text{mol}/\text{min}$ ) or high (235  $\mu\text{mol}/\text{min}$ ) amounts of  $\text{NH}_4\text{Cl}$  into the mesenteric vein of young sheep and assessed energy and protein

demand of the liver. Lobley *et al.* (1995) concluded that the detoxification of ammonia by the liver originating from the gastro-intestinal tract, incurs both energy and amino acid cost, lowering the nutrient availability for production. The hepatic output of urea in the ruminant is highly variable and any dietary manipulation that may alter ammonia production in the rumen could alter energy use by the liver.

### 1.3 Microbial growth

Due to microbial bio-synthesis ruminants can survive on diets that are virtually free of amino acids so long as there is sufficient non protein nitrogen present in the diet (Ammerman and Henry, 1985). Even when the diet contains the majority of nitrogen as protein, 50 to 80% of the nitrogen reaching the abomasum is likely to be of microbial origin (Hogan, 1975). When soluble energy and nitrogenous substrates are sufficient, an important determinant of relative microbial success (inter species competition for nutrients) is maximum growth rate. At such times an organism with the higher maximum growth rate is able to grow faster than an organism with a lower maximum growth rate. Russell and Baldwin (1978) found that maximum growth rates were dependent on energy source and Russell *et al.* (1979) observed growth rates to be influenced by the pH of the incubation medium.

Differences exist between microbes for growth potential from different energy substrates and small changes in nutrient availability for cellulolytics can result in large changes in yield per unit of substrate (Sniffen and Robinson, 1987). Russell and Sniffen (1983) incubated mixed rumen bacteria with timothy hay inoculum and protein synthesis increased by 16.4%, when additions of iso-valerate plus methyl butyrate were made. Similarly slight  $\text{NH}_3$  limitation has been seen to substantially decrease the yield of cellulolytic bacteria (Sniffen *et al.* 1983). In contrast, non cellulolytic bacteria are more directly influenced by carbohydrate availability (e.g. starch degradability, section 1.1.2) within a feedstuff which is itself influenced by processing (Sniffen and Robinson, 1987).

### 1.3.1 Yield of Adenosine triphosphate

The metabolism of rumen microbes during the initial stages of carbohydrate metabolism is predominately concentrated with the production of adenosine triphosphate (ATP) or similar molecules high in energy content, capable of participating in a wide variety of further exergenic reactions of cell synthesis (Hungate, 1988). The anaerobic conditions of the rumen make the complete oxidation of feedstuffs not possible and consequently yields of ATP (mols of ATP/mol energy source catabolized) are generally low (Nocek and Russell, 1988). As a result the amount of microbial biomass production per unit of energy source fermented, is substantially lower than that of aerobic bacteria. The preponderance of other volatile fatty acids over lactate as the end products of carbohydrate metabolism by rumen microorganisms enables more than 2 mols of ATP production per hexose molecule fermented, as opposed to just 2 when lactic acid is solely produced.

The relationship between ATP and synthesis of microbial biomass was first studied by Bauchop and Eldsen (1960). They compared the growth yields of several microorganisms with the theoretical amount of ATP that would be available and proposed the term  $Y_{ATP}$  (g bacterial DM/mol ATP) and assumed a constant value of 10.5. Isaccson *et al.* (1975) showed that the yield of ATP from mixed rumen bacteria under anaerobic conditions was in the order of 14 g per mol ATP and Stouthamer and Bettenhausen (1973) using rapidly growing cultures, in which maintenance costs were minimal, showed that the yield of anaerobic bacterial DM per mol of ATP to be 25 g. Stouthamer and Bettenhausen (1973) concluded that the efficiency of microbial growth depends on the



proportion of energy available for growth after maintenance and described this relationship with the following equation:

$$\frac{1}{Y(\text{ATP})} = \frac{1}{Y(\text{ATP})_{\text{max}}} + \frac{Me}{\mu}$$

where:

$Y(\text{ATP})$	=	g microbial DM/mol ATP
$Y(\text{ATP})_{\text{max}}$	=	maximum theoretical yield when ATP is used only for growth, expressed as g microbial DM/mol ATP
$Me$	=	Maintenance coefficient, mol ATP/g bacterial DM/h
$\mu$	=	specific growth rate (see Table 1.8)

### 1.3.2 Theoretical calculations of $Y_{\text{ATP}}$ values

Based on known metabolic pathways involved in biosynthesis of microbial cells, the amount of energy in the form of ATP required to form any one macromolecule can be calculated with reasonable accuracy. The values used by Hespell and Bryant (1979) are shown in Table 1.6. Knowing the ATP requirement for polymer formation, theoretical  $Y_{\text{ATP}}$  values for microbial cells can be calculated for any cell composition. Using these figures (Table 1.6) and basing their calculations on various observed microbial cell compositions, Hespell and Bryant (1979) estimated  $Y_{\text{ATP}}$  values for mixed rumen bacteria between 27 and 32.

Table 1.6. ATP requirement for the formation of microbial cells from monomers

Polymer	Monomer <sup>a</sup>	Monomer mol wt.	Biosynthesis <sup>a</sup>	ATP/ monomer synthesised
Protein	Amino acid	110	2 ATP:AA t-RNA 2 GTP:Peptide bond 1 ATP:mRNA turnover	5
RNA	Nucleotide Triphosphate	300	Ribose-5-P+ATP→PRPP PRPP+Base→NMP NMP+2ATP→NTP	5
DNA	Nucleotide Triphosphate	280	NTP→dNTP dUDP→dTTP	6
Lipid	Phosphatidyleth- anolamine + 2 palmitates	675	Glycerol + Acetate (via Malonyl Co.A)	33
Polysaccharides	Glucose	162	UDP-Glucose	2
Peptidoglycan	N-AcG-N-AcM	1000	UDP-Acetylglucosamine	14

Hespell and Bryant (1979)

<sup>a</sup>Abbreviations used - AA t-RNA, aminoacyl-transfer RNA; mRNA, messenger RNA; PRPP, phosphoribosyl-pyrophosphate; NMP, ribonucleoside monophosphate; NTP, ribonucleoside triphosphate; dNTP, deoxyribonucleoside triphosphate; N-AcG, N-acetylglucosamine; N-AcM, N-acetylmuramic acid.

Observed values of  $Y_{ATP}$  for anaerobically grown microorganisms are generally in the range of 10 to 15 g bacterial DM/mol ATP (Table 1.7), some what lower than theoretical estimations. It is unlikely that the firmly established biochemical equations used to calculate theoretical  $Y_{ATP}$  values are incorrect. The observed values pertain to an increased use of ATP for maintenance or to an over estimate of ATP production.

Table 1.7.  $Y_{ATP}$  values for anaerobically grown microorganisms

Organism	$^{\dagger}Y_{ATP}$	Reference
<i>Streptococcus faecalis</i>	10.9	Bauchop and Elsdon (1960)
<i>Saccharomyces cerevisiae</i>	10.2	Bauchop and Elsdon (1960)
<i>Escherichia coli</i>	9.4	Herandez and Johnson (1967)
<i>Sarcina ventriculi</i>	11.7	Stephenson and Dawes (1971)
<i>Bifidobacterium bifidum</i>	13.1	deVries and Stouthamer (1968)
<i>Ruminococcus albus</i>	11.5	Hungate (1963)
<i>Selenonomas ruminantium</i>	15.5	Hobson and Summers (1972)
<i>Lactobacillus casei</i>	20.0	deVries <i>et al.</i> (1970)
<i>Actinomyces naeslodii</i>	18.0	Buchanan and Pine (1967)
<i>Bacteroides ruminicola</i>	23.0	Howlett <i>et al.</i> (1976)
<i>Bacteroids fragilis</i>	19.6	Macy <i>et al.</i> (1975)

$^{\dagger}$  (g bacterial DM/mol ATP)

### 1.3.3 Factors influencing the production of the microbial biomass in the rumen

A summary of work completed on and possible reasons for differences in the theoretical and observed differences in  $Y_{ATP}$  are presented in Table 1.8. The various reasons and contributing factors are extensive. It should also be noted that various factors are related (eg rumen fluid pH and microbial enzyme activity) or are dependent (eg cell composition and futile cycles).

Table 1.8. Factors influencing the production of microbial biomass in the rumen.

Factor	Comments	Ref
Energy spilling reactions and futile cycles.	Substrate limited cultures (eg Sulphur, Phosphorus) have shown consumption of energy but with little microbial growth. Observations of increased heat production due to hexose catabolism but no associated microbial growth. Excess ATP dissipated as heat or used for synthesis of microbial reserve material (eg carbohydrate or lipid) changing cell composition.	1,2,3,4,5,6,7,8
Microbial enzyme activity.	Microbial biomass strives to produce maximum amount of ATP per unit of time. During periods of high rapidly available carbohydrate fermentative pathway is switched to produce lactate, yielding less ATP per unit of substrate.	7
Cell compositional changes	Bacterial cell composition can change dramatically under various conditions. Cell production with high proportions of protein and lipid requires more ATP for synthetic processes, lowering $Y_{ATP}$ .	6,9
Maintenance energy.	Energy needed for maintenance varies between organisms and environmental conditions. Of major importance is energy for active transport, phosphorylation, lysis and resynthesis of cells.	9,10
Specific growth rates or dilution rates.	As dilution rate increases fermenter residence time declines and therefore bacterial maintenance needs are reduced. Factors which influence residence time are; level of feeding, cell lysis and predation by protozoa, nitrogen recycling, dry matter intake to body weight ratio, water flux, molar proportions of volatile fatty acids.	11,12,13,14,15,16,17,18,19,22,23,24,25,26,27,28
Crude protein source and intake.	Very rapidly degradable protein sources are incorporated into microbial protein with less efficiency. Evidence to suggest that efficiency of microbial growth is enhanced by true protein sources.	28,29,30,31.
Methane production.	Most hydrogen produced in the rumen by the microbiota is used to reduce carbon dioxide to methane representing a loss of carbon (energy).	32.
Rumen pH.	As pH of rumen fluid falls protons may pass across bacterial membranes. The subsequent excretion necessary to maintain cellular neutrality is energy dependent.	33.

## References

1. Neijssel and Tempest (1976)
2. Forest and Walker (1965)
3. Stouthamer (1979)
4. Belaich *et al.* (1972)
5. Landzunski and Belaich (1972)
6. Nocek and Russell (1988)
7. Russell and Wallace (1988)
8. Mitchell (1961)
9. Hespell and Bryant (1979)
10. Russell and Baldwin (1979)
11. Owens and Goetsh (1986)
12. Maeng *et al.* (1976)
13. Van Soest (1982)
14. Evans (1981a)
15. Evans (1981b)
16. Sniffen and Robinson (1987)
17. Leng *et al.* (1984)
18. Sniffen and Robinson (1987)
19. Van Soest *et al.* (1982)
22. Chen *et al.* (1992)
23. Cole *et al.* (1976)
24. Owens and Isaccson (1977)
25. Warner and Stacey (1968)
26. Bennink *et al.* (1978)
27. Forbes and Barrio (1992)
28. AFRC (1992)
29. Maeng and Baldwin (1976)
30. Sehgal and Makkar (1994)
31. Robinson *et al.* (1987)
32. Wolin and Miller (1988)
33. Strobel and Russell (1986)

### 1.3.4 The efficiency of microbial growth

Bacterial growth in the reticulo-rumen requires the provision of ammonia, essential minerals, notably sulphur and phosphorous and carbohydrates to provide both an energy source and structural units (Smith, 1979). The rate of synthesis of microbes has been closely linked to the rate of fermentation of organic matter (Webster, 1987) and predicted yields of microbial protein defined as g of microbial nitrogen produced per kg organic matter fermented in the rumen (Verite *et al.* 1979; AFRC 1992), reflects the strong relationship that exists between carbohydrate and protein metabolism during microbial biomass synthesis.

Previous ration formulation systems (AFRC, 1990) have expressed the production of microbial protein (g) with single linear relationships depending on the metabolisable

energy content of the ration. The fixed ratio of 7.3 g microbial protein per mega joule of metabolisable energy (ARC, 1984) made no allowance for the level of feeding and the associated effects of rumen outflow rate on the amount of substrate fermented (see sections 1.1.2 and 1.2.6 for effective degradation). In addition, their reliance on metabolisable energy was over predicting energy supply to microbes because it included the energy content of fermentation products and fatty acids.

Recently it was suggested (AFRC 1990) that the yield of microbial protein should be predicted using a more dynamic approach related to rumen outflow rate, a function of the level of feeding. Recommended yields of microbial crude protein were 9 g/MJ FME at maintenance (rumen outflow of 0.02/h), 10 g/ MJ FME at twice maintenance (0.05/h) and 11 g/MJ FME at three times maintenance (0.08/h).

Where: 
$$FME^a = ME^b - (ME_{fat}^b + ME_{fermentation}^b)$$

<sup>a</sup> Fermentable metabolisable energy (MJ)

<sup>b</sup> Metabolisable energy (MJ),  $ME_{fat}^b$  = energy from fat,  $ME_{fermentation}^b$  = energy supplied by fermentation products.

The recommendations were modified further to overcome the discontinuity associated with this step approach by recognition of the exponential and asymptotic function between level of feeding and estimated yield of microbial crude protein (AFRC, 1992).

$$gMCP/MJ FME = 7 + 6(1 - e^{(-0.35L)})$$

Where: L = level of ME intake as a multiple of maintenance requirement.

Hvelpund (1986), in a review of existing data, showed that microbial growth efficiency was much more variable than this simplistic picture and found it ranged from 14 to 74 g of microbial nitrogen produced per kg organic matter apparently digested in the rumen (OMADR).

### **1.3.5 The rate of release of energy and protein in the rumen**

The prediction of microbial crude protein production per MJ of fermented metabolisable energy supply (AFRC, 1992) is basically a reflection of microbial needs for energy and nitrogen. In situations when nitrogen is not sufficient, uncoupled fermentation may occur and may result in substrate catabolism without microbial growth (see Table 1.8). Conversely, if nitrogen levels are excessive efficient incorporation into microbial protein may not be possible. Synchronization of nitrogen and energy degradation in the rumen is an approach proposed to increase bacterial growth rates and efficiency of nutrient utilization (Sniffen *et al.*, 1983; Nocek and Russell, 1988; Sinclair *et al.*, 1993,1995; Herrera-Saldana and Huber, 1989).

### **1.3.6 Description of synchrony**

Johnson (1976), in his review of the effects of varying forms of carbohydrate on non-protein nitrogen utilization, simplified the rates of fermentation of plant cell wall, starch and simple sugars into slow, medium or fast respectively (Fig 1.6a). Johnson (1976) went on to suggest that through the extensive degradation of protein, 80% of nitrogen passes through the rumen ammonia pool and for efficient energy use the pattern of ammonia production should reflect rates of carbohydrate fermentation (Fig 1.6b).



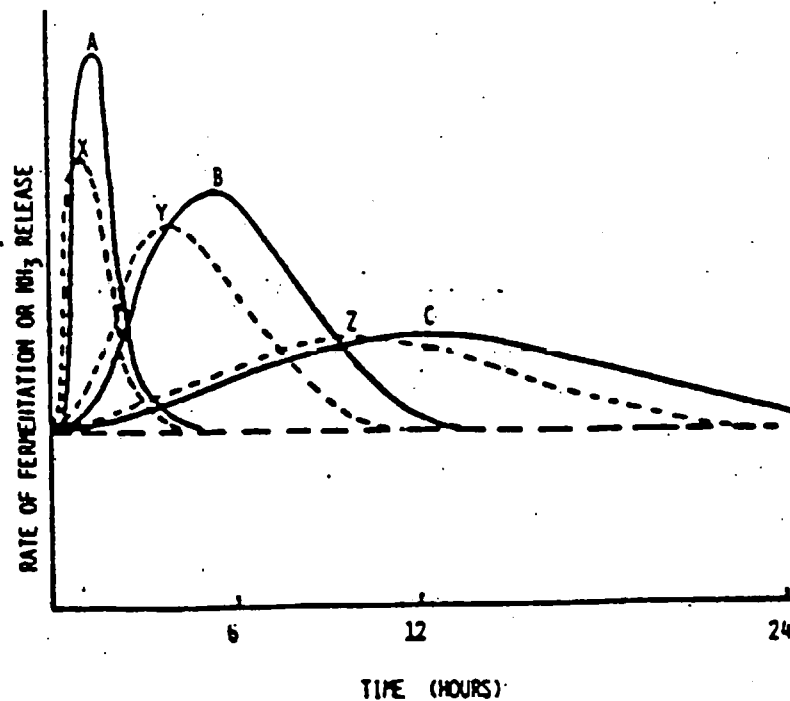
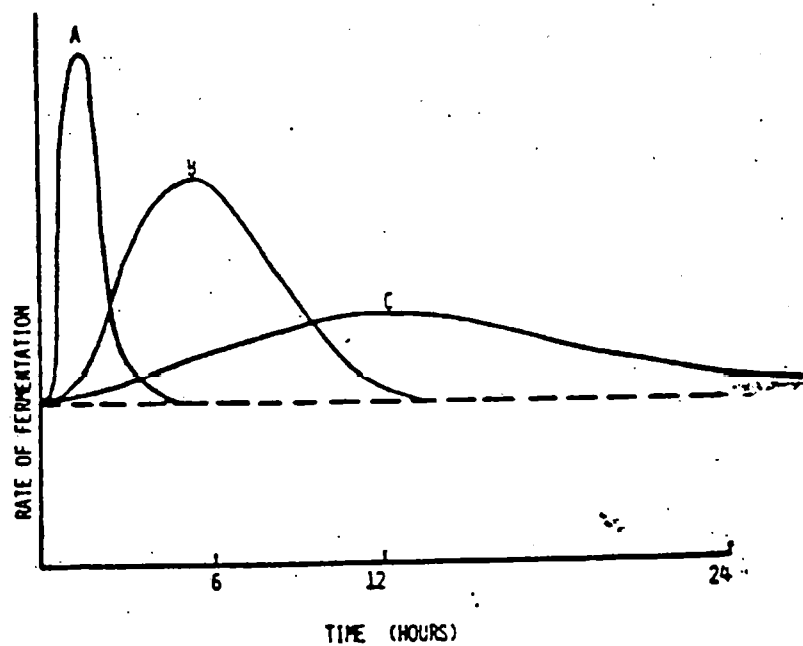


Fig 1.6. (a) Theoretical rumen fermentation rates over time of soluble sugars (A), starch and dextrins (B) and plant cell walls (C). Points on the curve might represent gas production or organic acid production at any point in time. (b) Theoretical rates of the same carbohydrates (A,B,C) and ammonia production according to degradable protein source (X,Y,Z). For example X could be urea, field peas or maize gluten meal, Y could be soya bean meal, groundnut oil cake or lupins and Z could be fishmeal or coconut oil cake (Johnson, 1976).

Conversely a “mismatch” of rates of fermentation of energy and protein would result in an asynchronous diet (Fig 1.7).

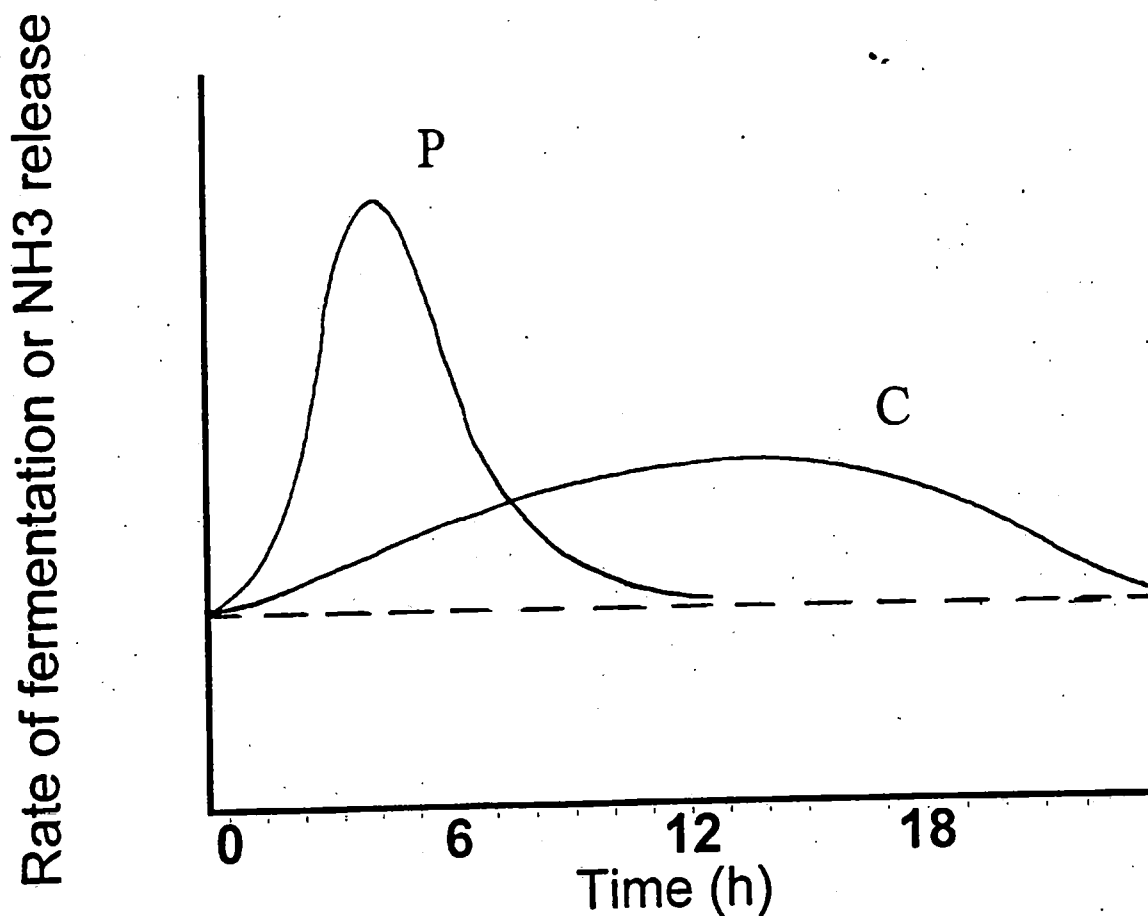


Fig 1.7. Theoretical rates of fermentation of an asynchronous diet. The carbohydrate source (C) could be based on wheat straw and maize and the protein source (P) could be based on field peas and barley distillers dark grains (Johnson, 1976).

### **1.3.7 Optimum ratio of nitrogen and energy**

Ration formulation (AFRC, 1992), outlined in section 1.3.4 suggests that the relationship between microbial crude protein produced synthesis and fermentable metabolisable energy supply is expressed by the equation below.

$$\text{gMCP/MJFME} = 7 + 6(1 - e^{(-0.35L)})$$

Where L = level of ME intake as a multiple of maintenance requirement.

### **1.3.8 Ratio of nitrogen to organic matter of various raw ingredients**

Table 1.9 presents calculated ratios of soluble nitrogen : soluble organic matter and potentially soluble nitrogen : potentially soluble organic matter for various raw ingredients. The effective degradation has been calculated as  $bc/c+r$ , assuming a transit time from the rumen of 0.08/h. Table 1.9 indicates that cereals that tend to be synchronous for their soluble content of nitrogen and organic matter are deficient in nitrogen to organic matter in their potentially degradable fractions. Conversely cereals that supply excess nitrogen in the soluble fractions approach a more synchronous release in their potentially degradable content of nitrogen and organic matter. Ingredients that are low in crude protein content (eg winter wheat straw) are deficient in nitrogen to organic matter in both the soluble and potentially degradable fractions, whereas ingredients that are high in protein (eg rape seed meal and fish meal) are constantly over supplying nitrogen. Table 1.9 indicates that grass silage is a feed that is particularly asynchronous in terms of the ratio of N:OM released for both the soluble and potentially degradable

fractions.

Table 1.9. Synchronicity of the soluble and insoluble but potentially degradable fractions of various ingredients. The optimum ratio is assumed as 28gN/kgOMTDR.

	SOM <sup>4</sup>	SN <sup>5</sup>	SN/SOM <sup>6</sup>	IOM <sup>7,10</sup>	IN <sup>8,10</sup>	IN/IOM <sup>9</sup>
Corn <sup>1</sup>	183.2	3.3	18.0	279	5.5	19.6
Milo <sup>1</sup>	14.7	0.6	37.2	291	6.1	20.8
Wheat <sup>1</sup>	600.6	19.8	32.9	202	3.9	19.3
Barley <sup>1</sup>	455.9	11.7	25.6	239	3.9	16.1
Oats <sup>1</sup>	772.8	19.8	25.6	47	0.3	7.0
Winter wheat straw <sup>2</sup>	103.3	1.7	16.6	115	0.3	2.3
Malt distillers dark grains <sup>2</sup>	398.6	23.6	59.1	185	5.2	28.1
Rape seed meal <sup>2</sup>	231.3	9.8	42.2	239	19.7	82.5
Fish meal <sup>2</sup>	275.5	43.7	158.6	33	4.7	144.0
Grass silage <sup>3</sup>	311.6	18.1	58.2	109	1.3	12.2

<sup>1</sup>Herrera-Saldana *et al.* (1990) - for OM, dry matter degradability coefficients used

<sup>2</sup>Sinclair *et al.* (1993)

<sup>3</sup>Makoni *et al.* (1991) - for OM, dry matter degradability coefficients used

<sup>4</sup>Soluble organic matter (g/kgDM)

<sup>5</sup>Soluble nitrogen (g/kgDM)

<sup>6</sup>Soluble nitrogen: Soluble organic matter (g/kg)

<sup>7</sup>Insoluble but potentially degradable organic matter (g/kgDM)

<sup>8</sup>Insoluble but potentially degradable nitrogen (g/kgDM)

<sup>9</sup>Insoluble degradable organic matter : Insoluble degradable nitrogen (g/kg)

<sup>10</sup>Calculated as  $bc/c+r$ , where  $r = 0.08$ .

### 1.3.9 *In vitro* experiments to access the effects of different rates of energy and nitrogen degradation on microbial growth

Newbold and Rust (1992), using batch culture techniques investigated the effects of a synchronous supply of isonitrogenous and isoenergetic nutrients on rumen bacterial

growth. Synchrony was achieved by supplying hourly additions of an aqueous glucose and urea mixture at a rate of 26 mgN/g glucose to strained rumen liquor plus buffer. To mimic an asynchronous feeding situation the ratio of nitrogen to glucose was increased exponentially over time. In an additional experiment, large and small corn particles were used as slow and fast energy sources respectively, and samples of untreated or a digest of soya bean meal as slow and fast nitrogen sources respectively, arranged in a 2 X 2 factorial design. During times of limited nitrogen supply in both experiments bacterial growth was limited. Even though total bacterial yield was equal after a 12 h incubation the time taken to achieve this yield was higher for bacteria growing under asynchronous conditions (Fig 1.8).

Newbold and Rust (1992) concluded that transient deficits of nitrogen had no lasting effect on bacterial growth using batch culture techniques. The reduction in growth from 7 h onwards (Fig 1.8) in bacteria supplied with a synchronous supply of nutrients may have reflected end point inhibition. Limitations of nitrogen supply have been associated with increased heat production by the biomass (Russell and Wallace, 1988) and changes in bacterial cell composition (Landzunski and Belaich, 1972) both of which may affect the efficiency of bacterial energy use. The effects of short term deficits or excesses of nitrogen supply on microbial efficiency are difficult to study accurately *in vitro* because ammonia absorption and recycling, plus the energy cost of urea synthesis cannot be simulated.

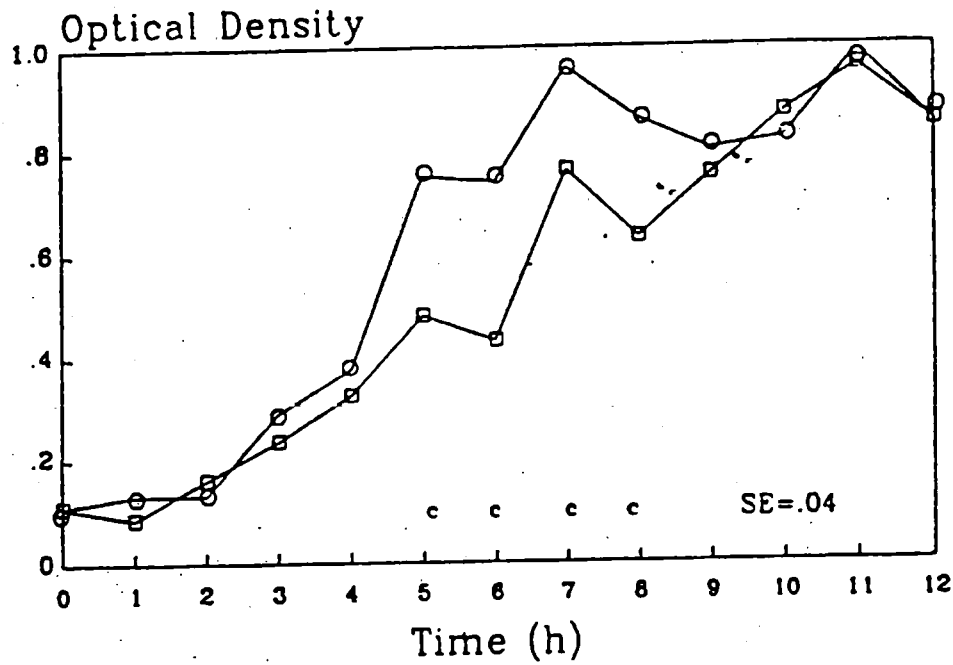


Fig 1.8. Bacterial population size according to particle density supplied with either a synchronous (○) or asynchronous (□) amount of urea and glucose. c =  $p < 0.001$ . Newbold and Rust (1992).

Unlike Newbold and Rust (1992), Henning *et al.* (1993) found no significant effects of a synchronous release of energy and nitrogen on microbial metabolism using batch culture techniques, and concluded that a fast supply of energy of more importance to promote higher bacterial efficiencies. Similarly Mansfield *et al.* (1994) and Hussein *et*

*al.* (1991) using continuous culture techniques observed no improvement in ruminal fermentation due to synchrony but, because of the significance of main effects, found bacterial growth to be more likely limited by either supply of energy or nitrogen alone.

### **1.3.10 *In vivo* experiments to access the effects of different rates of energy and nitrogen degradation on microbial growth**

#### **1.3.10.1 Infusion techniques**

The rapid production of ammonia in the rumen from the substantial proportion of soluble non protein nitrogen present in grass silage coupled with the large content of slowly degradable plant cell walls gives rise to a particularly asynchronous ruminant feedstuff (Rooke *et al.* 1987). As a result synchronization of energy and nitrogen release in the rumen has been regarded as being particularly relevant to diets based on grass silage. Khalili and Huhtanen (1991a; 1991b) found significant decreases in the concentration of rumen ammonia in animals continuously infused with sucrose when fed diets containing grass silage. The authors also observed significant increased flows in microbial nitrogen to the duodenum and non significant increases in the efficiency of nitrogen incorporation into rumen microbes (Table 1.10).

Khalili and Huhtanen (1991a;1991b) found no improvement in microbial growth and efficiency when a pulse dose of sucrose was used as a supplement to grass silage. They reported decreases rumen pH and neutral detergent fibre degradation and increased



lactate concentrations in rumen fluid in animals receiving the pulse doses of sucrose and concluded that close synchronisation of energy and nitrogen release was not required for maximum fixation of rumen ammonia. However, Chamberlain and Choung (1995) suggested that very rapid rates of fermentation promoted by some sugars given in large doses may have adverse effects on microbial growth due to end product suppression.

Rooke *et al.* (1983;1985) suggested that microbial nitrogen synthesis was restricted in silage diets due to a lack of ammonia nitrogen post prandial. However, in a subsequent experiment Rooke *et al.* (1987) found significant increases in microbial protein production and efficiency above controls when either glucose or glucose plus casein was infused into the rumen of cattle fed grass silage based diets. There was no such increase in animals infused with just urea or casein. Rooke *et al.* (1987) concluded that additional non protein-, amino- or peptide nitrogen *per se* was not an important limiting factor in improving the efficiency of microbial protein synthesis in silage based diets.

Table 1.10. Effects of various infusates on rumen metabolism

Infusate	Rumen pH		Rumen NH <sub>3</sub> (mmol/l)	MicN at the duodenum (g/d)	Microbial efficiency <sup>d</sup>	Ref
	Min	Mean				
None	6.0	6.28	12.57 <sup>a</sup>	71.8 <sup>a</sup>	25.5	1
Continuous sucrose (1 kg/d)	5.7	6.12	5.21 <sup>b</sup>	104.5 <sup>b</sup>	30.8	
Pulse dose sucrose (1 kg/d)	5.4	6.03	7.07	89.8	27.6	
Water	na	6.80	3.00	63 <sup>b</sup>	22	2
Casein	na	6.80	5.80 <sup>b</sup>	75	25	
Urea	na	6.80	4.80 <sup>b</sup>	68 <sup>b</sup>	25	
Glucose	na	6.80	1.6 <sup>a</sup>	81 <sup>a</sup>	27	
Glucose + casein	na	6.70	2.3	109 <sup>a</sup>	38	

means with different superscripts in the same column within the same experiment are significantly different ( $p < 0.05$ )

- References
1. Khalili and Huhtanen (1991a) - <sup>a</sup>gN/kgOMTDR
  2. Rooke *et al.* (1987) - <sup>a</sup>gN/kgOMADR

### 1.3.11 Starch versus sugars as supplements for grass silage

Chamberlain *et al.* (1993) supplemented grass silage diets with either 200 g/d of simple sugars or raw maize starch (Table 1.11). All four sugar supplements significantly increased microbial protein production compared to silage alone with fructose promoting the highest microbial yields compared to starch.

Table 1.11. Effects of supplementation of grass silage with starch and a number of different sugars.

	Supplement to grass silage (200 g/d)					
	None	Sucrose	Lactose	Xylose	Starch	Fructose
Rumen pH						
Mean	6.43	6.34	6.40	6.16	6.25	6.31
Min	6.04	5.72	6.05	5.97	5.99	5.74
NH <sub>3</sub> -N (mg/l)						
Mean	255	157	158	180	288	164
Max	354	240	234	240	313	233
Microbial Protein (g/d)	64 <sup>b</sup>	93 <sup>a</sup>	89 <sup>a</sup>	82 <sup>a</sup>	74	86 <sup>a</sup>

Means with different superscripts in the same row are significantly different ( $p < 0.05$ )

Source: Chamberlain *et al.* (1993)

The authors concluded that sugars, particularly sucrose, were more efficiently used by microbes resulting in a greater capture of silage nitrogen than the raw maize starch. Salter *et al.* (1983) suggested that different fermentable carbohydrates will promote different rumen populations of micro flora and that starch will greatly increase the number of protozoa. Higher numbers of rumen protozoa have been associated with greater rumen recycling of nitrogen ultimately decreasing the daily yield of microbial nitrogen (Russell and Wallace, 1988).

Chamberlain and Choung (1995) also increased the rate of fermentation of starch by cooking it and found an increase in microbial nitrogen yield (Table 1.12). Not all soluble sugars promoted high microbial yields when used to supplement grass silage (Table 1.12) and there was evidence that there were intrinsic differences in microbial utilization of different sugar sources.

Table 1.12. Effects of different carbohydrate supplements to grass silage on the synthesis of ruminal microbial protein (g/kg added carbohydrate).

Carbohydrate	gMicN/kg added carbohydrate	ref.
Sucrose	33	Huhtanen (1987)
Xylose	23	
Glucose syrup	28	Rooke <i>et al.</i> (1987)
Sucrose	21	Kahalili and Huhtanen (1991)
Sucrose	23	Chamberlain <i>et al.</i> (1993)
Lactose	22	
Xylose	15	Chamberlain and Choung (1995)
Fructose	18	
Maize starch uncooked	9	Chamberlain and Choung (1995)
Maize starch cooked	21	
Sucrose	25	Chamberlain and Choung (1995)
Barley starch	20	
Barley starch cooked	28	

Cited in Chamberlain and Choung (1995)

### 1.3.12 Other *in vivo* experiments measuring the effects of the rate of energy and nitrogen release in the rumen on microbial metabolism

Nocek and Russell (1988) argued that microbial growth is ultimately dependent on the

relative availabilities of carbohydrate and protein in the rumen and ration formulation should include a description of the feed in these terms. Their theoretical calculations predicted that because of times of deprivation of energy and nitrogen caused by asynchronicity, microbial yield and efficiency could be increased using ingredients that were more complimentary in their rates of release of energy and nitrogen. To test this theory various feeding experiments have been undertaken and a sample of results are presented in Table 1.13.

McCarthy *et al.* (1989) fed corn (maize) and barley and fish meal and soya bean meal as slow and fast energy and nitrogen sources respectively *ad libitum* to cattle (Table 1.13). The authors could find no beneficial effects of a more synchronous degradation but a fast rumen release of nitrogen supported a higher production of microbial nitrogen (g/d). Hussein *et al.* (1991), using a similar experimental design but with diets offered at a restricted level of feeding (1 kg /d) to sheep, found microbial flow and efficiency of production was greater when diets promoted a fast release of nitrogen rather than when diets were synchronous (Table 1.13). Similarly, Herrera-Saldana *et al.* (1990) using barley supplemented with cottonseed meal to promote a fast release of energy and nitrogen (FEFN) in the rumen of cattle, found it stimulated a greater microbial efficiency compared to an asynchronous or slow synchronous ingredients (Table 1.13). Henning *et al.* (1993), using infusion techniques, could find no benefit from synchrony and concluded that it was more important to provide an even supply of energy. However these conclusions could have been confounded due to end product depression caused by the pulse dose of sugar, as indicated by Russell and Wallace (1988). Additionally the feeding levels used were such that rumen ammonia levels never fell to a level sufficient to inhibit

microbial growth.

Table 1.13. Effects of various ingredients chosen to promote different rates of fermentation of energy and nitrogen in the rumen and their effects on microbial metabolism.

	Diets <sup>a</sup>				Main effects		Ref
	SESN	SEFN	FEFN	FESN	E	N	
Microbial N (g/d)	259	294	310	282	NS	*	1
	342	378	480	422	NS	NS	2
	9.1	13.8	15.5	12.1	NS	*	3
Microbial efficiency (gN/kgOMTDR)	31.2	35.2	31.2	30.9	NS	NS	1
	35.7 <sup>b</sup>	35.4 <sup>b</sup>	45.3 <sup>a</sup>	37.1 <sup>b</sup>	*	NS	2
	28.7 <sup>b</sup>	36.6 <sup>a</sup>	37.6 <sup>a</sup>	20.3 <sup>b</sup>	NS	*	3

Means in the same row with different superscripts are significantly different ( $p < 0.05$ )

<sup>a</sup>SESN - slow energy slow nitrogen, SEFN - slow energy fast nitrogen, FEFN - fast energy fast nitrogen, FESN - fast energy slow nitrogen.

#### References

1. McCarthy *et al.* (1989) - cattle
2. Herrera-Saldana *et al.* (1990) - cattle
3. Hussein *et al.* (1991) - sheep

The above experiments, either *in vitro* or *in vivo* attempted to evaluate the effects of synchronous or asynchronous supplies of energy and nitrogen to the rumen on microbial metabolism. Henning *et al.* (1993) chose infusion techniques because they felt that any result obtained using different ingredients to achieve synchrony would be confound by ingredient effects. All infusion experiments described above used soluble sugar sources as infusates to induce fast or slow nutrient release. Efficient microbial growth *in vivo* relies on the synergistic effects of the entire microbial population and therefore supply of solely soluble sugars may confound results by favouritism toward the soluble sugar utilizers.

The variability and lack of conclusive evidence for the existence of rumen synchronicity could be attributed to the lack of objectively employed in dietary manipulation. Table 1.3 indicated that different batches of the same feed possess different degradability coefficients and will alter the attainable synchrony or asynchrony. Sinclair *et al.* (1993; 1995) produced a number of diets based on the observed *in situ* degradability of the ingredient feeds. Sinclair *et al.* (1993) characterised the degradability values of 5 different ingredients *in sacco*. Using a computer program two diets were formulated, one to be synchronous and one to be asynchronous with respect to their hourly release of organic matter and nitrogen in the rumen. Observed microbial efficiencies were up to 14% higher in sheep fed synchronous diets (Table 1.14). Using the same ingredients and computer program Sinclair *et al.* (1995) formulated two rations with similar carbohydrate composition but to be either synchronous or asynchronous. Microbial efficiencies were again increased in sheep fed the synchronous rations (Table 1.14) although the differences did not always achieve statistical significance (Sinclair *et al.* 1993; 1995).

Table 1.14. The effect of synchronous or asynchronous supply of hourly organic matter and nitrogen in the rumen of sheep.

	Syn	Asy	s.e.	Reference
Microbial efficiency (gN/kgOMTDR)				
based on [ <sup>3</sup> H]leucine	30.8	27.0	2.43	Sinclair <i>et al.</i> (1993)
	30.7	27.5	0.603	Sinclair <i>et al.</i> (1995)
based on cytosine	27.4	24.3	2.74	Sinclair <i>et al.</i> (1993)
	26.9	23.9	0.228	Sinclair <i>et al.</i> (1995)

### **1.3.13 The effect of rate of degradation of energy and protein on whole animal performance**

Despite the relatively large amount of work that has been covered concerning the effects of various rates of degradation of energy and nitrogen on rumen metabolism, very little work has been completed measuring the effects of rumen synchrony on host metabolism, especially growth. However experiments have been completed which compare different levels and sources of energy and protein and a number are summarized in Table 1.15.



Table 1.15. Effects of various rates of degradation of energy and protein in the rumen on host productive performance.

Factor	Comments	Ref
Rate of protein degradation	Level of crude protein and rate of degradation had no effect on growth and carcass characteristics of male and female lambs	1
Protein and energy interactions	Lambs fed diets varying in the quantity of rumen degradable energy and nitrogen. Significant effects of protein and energy interactions on live weight gains.	2
Supplementation of silage with various energy and protein feeds	Growth and carcass characteristics of steers fed silage supplemented with varying amounts of rape seed meal and molasses. Live weight gain increased by rape seed meal. No effect of molasses.	3
Rate of energy degradability	Formaldehyde treated barley (slow energy) compared with untreated barley (fast energy). Slow energy reduced kidney fat. Different protein sources altered NDF degradability.	4
Supplementation of straw with different sources of energy and protein	Wethers fed urea treated straw. Interaction between energy and protein supplements on feed conversion efficiencies suggested responses to protein depends on supplementation with energy.	5
Varying energy and protein amounts and sources to ewes and effect on lamb growth	No interaction between energy and nitrogen source on lamb performance. Milk composition not influenced by protein source.	6
Protein and energy effects on endocrinology	Higher supplies of protein decreased growth hormone and increased insulin. Metabolic supply of energy and nitrogen positively correlated with insulin. Varying energy and nitrogen supply to host altered fatty acid synthesis.	7

#### References

1. Beauchemin *et al.* (1995)
2. Gorgulu *et al.* (1994)
3. Petit *et al.* (1994)
4. McAllister *et al.* (1992)
5. Cronje and Weites (1990)
6. Sheenan and Hanrahan (1989)
7. Waghorn *et al.* (1987)

Lactation studies to examine the effects of varying the rate of release of ruminal energy and protein have produced varied results. Robinson and McQueen (1994) altered the rate of supply of protein in multiparous Holstein cows by feeding the protein source in different amounts and frequency but to supply similar amounts of energy and protein

over a 24 h period. Milk yield and composition were not effected by treatment. However the protein supplement only accounted for 20 % of dietary nitrogen intake, perhaps an amount insufficient to significantly alter rumen ammonia concentrations above the basal ration. Additionally, based on *in sacco* degradability data, it was calculated that at no time was microbial growth limited by lack of degradable protein (Robinson and McQueen, 1994).

Casper and Schingoethe (1989) compared combinations of corn and barley (slow and fast energy) with soya bean meal and urea (slow and fast nitrogen) when fed to dairy cows. The authors reported no interaction between energy and nitrogen on milk composition but that yield of fat corrected milk (4%) was significantly lower for cows fed diets predicted to be synchronous. Herrera-Saldana and Huber (1989) formulated diets varying in their pattern of release of energy and nitrogen based on feedstuff characterisation. Rapid and slowly degradable starch and protein sources were barley, milo, cotton seed meal and brewers dried grains respectively. Matching the rate of energy and protein degradation tended to increase fat corrected milk yields (3.5%) and barley plus cottonseed meal (FEFN) significantly increased milk production per day (Table 1.16):

Table 1.16. Effect of rate of digestion of energy and nitrogen in the rumen on milk production in cattle

	Diets <sup>a</sup>				Main effects <sup>b</sup>		
	SESN	SEFN	FEFN	FESN	E	N	I
From Herrera-Saldana and Huber (1989)							
Yield (kg/d)	34.6 <sup>b</sup>	34.2 <sup>b</sup>	37.4 <sup>a</sup>	34.9 <sup>b</sup>	na	na	na
Fat (g/kg)	36 <sup>a</sup>	34 <sup>a</sup>	31 <sup>b</sup>	29 <sup>b</sup>	NS	*	NS
Protein (g/kg)	28	30	29	30	NS	NS	NS
From Aldrich <i>et al.</i> (1993)							
Bacterial N (g/d) at duodenum	237	234	262	214	NS	*	*
gN/kgOMTDR	17.8	15.2	17.9	14.5	NS	NS	**
Milk (kg/d)	39.6	39.5	39.3	38.8	NS	NS	NS
Fat content (g/kg)	33.8	34.6	32.6	34.0	NS	NS	NS
Protein content (g/kg)	29.8	30.4	30.8	30.5	**	*	NS

Means in the same row with different superscripts are significantly different ( $p < 0.05$ )

<sup>a</sup>SESN - slow energy slow nitrogen, SEFN - slow energy fast nitrogen, FEFN - fast energy fast nitrogen, FESN - fast energy slow nitrogen.

<sup>b</sup>E - Energy, N - Nitrogen, I - interaction, na - not available

Aldrich *et al.* (1993) found that multiparous cows offered synchronous diets had significantly higher bacterial flow rates at the duodenum and efficiency of microbial nitrogen capture. Although there was no significant effect on milk yield or composition (Table 1.16) diets that promoted rapid rates of release of energy and nitrogen (FEFN) tended to increase milk protein content. It is also interesting to note that a synchronous release of energy and nitrogen tended to reduce milk fat content and significantly decreased milk fat yield.

Work designed to evaluate the effects of synchrony on lactation has taken a factorial approach with two rates of carbohydrate release. Using this approach may confound the effects of asynchrony as the choice of energy yielding substrate for the rumen could alter

the ratio of keto- and glucogenic precursors thus affecting the partitioning of nutrients. Similarly differences in starch degradability will alter levels of bypass starch which could affect milk protein content and yield. These factors may or may not alter lactational response but it becomes harder to evaluate effects of purely synchrony in this manner.

#### **1.4 The present study**

Evidence for effects of a synchronous release of energy and protein in the rumen on whole animal performance is lacking, inconclusive and warrants further investigation. Variation in the rumen degradability characteristics of raw materials makes it difficult to rely on published data to formulate rations which accurately vary in the rate and extent of protein and energy release in the rumen. The experiments described in this thesis investigated the effects of feeding diets predicted from the sum of *in situ* degradability data to supply similar levels of metabolisable nutrients, but to vary in their rumen availability of organic matter and protein on the performance of growing lambs and lactating ewes.

## **CHAPTER 2**

### **2. MATERIALS AND METHODS**

#### **2.1 Routine analysis**

##### **2.1.1 Dry matter**

Sub-samples of all feed ingredients, concentrate rations, grass silage and residues from synthetic bags were analysed for dry matter content (g/kg) by drying in an oven at 80°C to constant weight.

##### **2.1.2 Organic matter**

Approximately 1 g of dried material was accurately weighed into porcelain crucibles and then heated to 450°C in a muffle furnace for 16 h. Samples were then cooled in a desiccator to room temperature and reweighed. The content of organic matter was calculated as 1000-ash (g/kgDM) (AOAC, 1980).

##### **2.1.3 Ether extract**

Approximately 2-3 g of ground (1mm screen) fresh material was accurately weighed into cellulose extraction thimbles which were then plugged with defatted cotton wool. Total fat was then extracted by boiling samples in 25 ml of petroleum ether for 30 mins.

Samples were then allowed to rinse for 15 mins. Final traces of the petroleum ether was allowed to evaporate in a fume cupboard. The fat content was then calculated as:

$$\text{Ether extract (g/kgDM)} = \frac{\text{Weight of fat (g)}}{\text{Weight of sample (gDM)}} \times 1000$$

#### **2.1.4 Crude protein**

Samples of approximately 0.5 - 1.0 g of dried material from feed and carcass samples or residues from synthetic bags were analysed by automated kjeldahl procedure (Tecator). Samples were accurately weighed into digestion tubes containing two kjeltab catalyst tablets and then boiled in H<sub>2</sub>SO<sub>4</sub> (6N, 14ml) at 400°C for 45 mins. Samples were allowed to cool and then deionised water was added (75 ml). Crude protein content (total N x 6.25) was then estimated via back titration using HCl (0.2 M) as the titrant.

#### **Non structural carbohydrates**

##### **2.1.5 Water soluble carbohydrates**

The soluble carbohydrate content of the raw ingredients was determined by the method of MAFF (1981). Exactly 0.2 g of dried ground material was weighed into a 250 ml bottle and 200 ml of distilled water was added. The bottle was capped and then shaken for 1 h. The extract was then filtered (Whatman No. 1 filter paper) into a 250 ml conical

flask except for the last 2 ml of extract which was discarded. Filtrate (2 ml) was pipetted into a boiling tube and 10 ml of anthrone reagent (380 ml of concentrated sulphuric acid added to 165 ml of distilled water to which 0.5 g of thiourea and 0.5 g of anthrone were added and the solution gently stirred until the thiourea and anthrone dissolved) was added. Tubes were stoppered and the contents thoroughly mixed. Samples were then placed in a water bath at 95°C for 20 min. Tubes were then removed from the water bath and placed on ice to reduce sample temperature as quickly as possible. Absorption was read at 620 nm using a Beckman DU600 spectrophotometer in a 1 cm<sup>3</sup> cuvette. A standard curve was produced using 2 ml of 0, 0.04, 0.08, 0.12, 0.16 and 0.20 mg/ml of glucose added to 10 ml anthrone reagent and following the procedure above.

#### **2.1.6 Starch**

Starch was determined according to the method of Wainman, Dewey and Boyne (1981). Approximately 0.2 g of finely ground sample was accurately weighed into a 100 ml centrifuge tube and 20 ml of hot (85°C) ethanol (85%) added. Samples were mixed using a whirlimixer and then allowed to stand for 5 mins. Samples were then whirlimixed again and centrifuged at 2500 rpm for 10 mins. Supernatants were carefully decanted and discarded. To the residue 20 ml of hot (85°C) ethanol (85%) was added and the procedure repeated. Distilled water (20 ml) was added to the residue and samples were whirlimixed, loosely stoppered and placed in a boiling water bath for 2 h. Samples were then allowed to cool to below 40°C before 50 ml of acetate buffer (27.2 g sodium acetate-trihydrate and 12 ml glacial acetic acid in 1 litre) was added and mixed. Amyloglucosidase enzyme suspension was prepared by adding 0.5 g amyloglucosidase to 50 ml acetate buffer and



2 ml added to each tube. Two drops of toluene were added and the tubes incubated overnight at 50-55°C.

Tube contents were made up to 100 ml with distilled water, allowed to cool to room temperature and then filtered through a Whatman 541 filter paper. The filtrate was diluted 1:10 (for samples containing 20-60% starch; for those outside this range appropriate dilutions were made) and 0.2 ml was transferred into a 10 ml test tube. In addition 0.2 ml of glucose standard was placed into another 10 ml test tube. Standard solution was taken from a Boehringer Test Kit ("GOD-Perid" colorimetric test kit for glucose estimation). Reagent blank (0.2 ml) and distilled water (0.2 ml) were added to a third and fourth tube respectively. To each tube was added 5.0 ml of buffer/enzyme/chromogen (supplied with the test kit). Tubes were stoppered and mixed thoroughly using a whirlimixer. Tubes were allowed to stand at room temperature for 25-50 min (taking care to ensure that tubes were not exposed to direct sunlight) and the absorbency read at 610 nm using a Beckman DU600 spectrophotometer and a 1 cm<sup>3</sup> cuvette.

The content of glucose (µg) in 0.2 ml aliquot of diluted sample was calculated as:

$$\frac{\text{Absorption of sample} - \text{Absorption of reagent blank}}{\text{Absorption of standard}} \times 18.2$$

The percentage of glucose in the sample was calculated as:

$$\frac{\mu\text{g glucose}}{\text{Sample weight}} \times 0.05 \times \text{dilution rate}$$

Starch was calculated as 0.9 x glucose.

## **Structural carbohydrate analysis**

Fibre analysis was determined according to the method of Goering and Van Soest (1970) using Fibretech (Tecator) apparatus (Foss UK 1020).

### **2.1.7 Amylase Neutral detergent fibre (NDF)**

Samples (0.5 g) of ground, dried material were accurately weighed into a previously weighed crucible. The crucible was then secured into place in the Fibretec apparatus (Tecator, Foss UK 1020) and 25 ml of cold neutral detergent solution (93 g disodium ethylene diamine tetra-acetate dihydrate (EDTA) and 34 g sodium borate dissolved in distilled water using gentle heating. To this 150 g sodium lauryl sulphate and 50 ml 2-ethoxy ethanol were added. In a separate flask 22.8 g anhydrous disodium hydrogen phosphate was dissolved in distilled water. The 2 solutions were mixed and then diluted to 5 l. The pH was adjusted to between 6.9 and 7.1) was dispensed into each sample. Also, 0.5 ml of anti-foaming agent (octanol) was added.

The samples were boiled for 30 mins after which samples were washed (filtered) 3 times using 20 ml hot (80°C) distilled water. Filtrates were discarded. Warm (55°C) deionised water was then added to the residues and 2 ml  $\alpha$ -amylase solution (2.2 g of amylase (BDH) was dissolved in 99 ml of distilled water. This was then filtered and 11 ml 2-ethoxy ethanol was added to the filtrate), prepared daily was added to each sample. Samples were then mixed and allowed to stand for 30 mins and then washed 3 times with hot (80°C) deionised water and finally with 100% acetone (20 ml). Crucibles, containing

residues were then removed from the fibretec apparatus, and dried at 100°C overnight in an oven. Crucibles were then cooled in a desiccator and reweighed. Samples were then ashed at 550°C for 4 h, cooled in a desiccator and reweighed.

NDF content (g/kgDM) was calculated as:

$$\frac{\text{residue weight (g)} - \text{ash content (g)}}{\text{Sample weight (gDM)}} \times 1000$$

#### **2.1.8 Acid detergent fibre (ADF)**

Samples (1 g) of ground dried material were accurately weighed into a previously weighed crucible. The crucible was then secured into place in the Fibretec apparatus (Tecator, Foss UK 1020). To each sample was added 100 ml of acid detergent reagent (20 g of cetyl trimethylammonium bromide (CTAB) dissolved in sulphuric acid (1 M)) and then boiled for 60 min, filtered and washed 3 times using hot (80°C) water (20 ml) and once with acetone (20 ml). Crucibles, containing residues were then removed from the fibretec apparatus, and dried at 100°C overnight in an oven. Crucibles were then cooled in a desiccator and reweighed. Samples were then ashed at 550°C in a muffle furnace (Fisons) for 4 h, cooled in a desiccator and reweighed.

ADF content (g/kgDM) was calculated as:

$$\frac{\text{residue weight (g)} - \text{ash content (g)}}{\text{Sample weight (gDM)}} \times 1000$$

### **2.1.9 Acid detergent lignin (ADL)**

Samples of ADF residue were digested with acid detergent reagent (section 2.1.11). Prior to heating to 550°C, 25 ml of 72% (v/v) of concentrated sulphuric acid was added. Samples were incubated at room temperature for 3 h and mixed every hour. Samples were then filtered and washed 3 times with hot (80°C) water (20 ml) and finally with acetone (20 ml). Crucibles, containing residues were then removed from the fibretec apparatus, and dried at 100°C overnight in an oven. Crucibles were then cooled in a desiccator and reweighed. Samples were then ashed at 550°C in a muffle furnace (Fisons) for 4 h, cooled in a desiccator and reweighed.

ADL content (g/kgDM) was calculated as:

$$\frac{\text{residue weight (g)} - \text{ash content (g)}}{\text{Sample weight (gDM)}} \times 1000$$

### **2.1.10 Acid detergent insoluble nitrogen (ADIN)**

A kjeldahl analysis was carried out using a Tecator auto analyser as described in section 2.1.4 on the residue obtained after an ADF procedure prior to heating to 550°C (section 2.1.11).

### **2.1.11 Neutral cellulase gamanase digestibility (NCGD)**

The metabolisable energy content (MJ/kgDM) of the raw ingredients feeds were

determined by a neutral cellulase gamanase digestibility test (NCGD; MAFF, 1993).

### **2.1.12 Rumen fluid**

#### **2.1.12.1 Extraction of rumen fluid**

Rumen fluid samples were taken from animals using a stomach tube. Animals were removed from their pens, constrained so that movement was minimised. A gag was placed in the sheep's mouth (to prevent chewing) and a flexible tube was pushed gently down the oesophagus into the reticulorumen. Rumen fluid was then extracted (approx. 125 ml) using a vacuum pump. The first 25 ml was discarded. The collection vessel and flexible tube were washed in warm water between collections. Rumen fluid was immediately strained through 2 layers of muslin and the pH of the rumen fluid recorded using a pH electrode. Rumen fluid samples were acidified using sufficient concentrated HCl so that the final pH was less than 3 and the acidified samples were then frozen prior to analysis.

#### **2.1.12.2 Rumen volatile fatty acid (VFA) concentration**

Samples (10 ml) of strained rumen fluid (SRF) were centrifuged at 25,000 g for 20 mins. Supernatants were carefully decanted, filtered through cellulose filter paper (Whatman, Cellulose Nitrate, 0.2  $\mu$ m) and residues discarded. To the filtrate (0.9 ml) was added 0.1 ml of phenol (250 mM) as an internal standard. Volatile fatty acid concentrations were estimated using Gas Liquid Chromatography (Perkin-Elmer 8500 filtered with a AS3800

auto-sampler). The column was supplied by J and W Scientific, Fisons (FFAP, 30 m long and an internal diameter of 0.25 mm (narrow bore)), initial oven operating temperature was 110°C rising to 200°C at a rate of 10°C/min after 17.0 mins. The total running time was 31 mins. Retention times of individual VFA in rumen fluid were calculated with reference to a calibration curve of an external standard (174.8 mmol acetic acid, 106.9 mmol propionic acid, 10.78 mmol iso-butyric acid, 54.4 mmol butyric acid, 9.1 mmol iso-valeric acid, 9.2 mmol valeric acid, 8.0 mmol caproic acid per litre).

## **CHAPTER 3**

### **3. IN SITU CHARACTERIZATION OF FEED COMPONENTS AND CALCULATION OF THE HOURLY RELEASE OF NUTRIENTS IN THE RUMEN**

#### **3.1 Introduction**

The description of the characterisation procedure for the silage used in the lactation trial is described in chapter 5. However the proximate analysis data and fibre content appear in the following Tables.

The formulation of rations for ruminants are currently prepared on a daily basis (AFRC, 1993; Vèritè *et al.*, 1979) with no particular attention paid to the pattern of supply of nutrients to the rumen population. Sinclair *et al.* (1995) observed that diets formulated using their computer program (Synchrony Index for the Rumen Environment, S.I.R.E.) to be synchronous in relation to the hourly release of energy and nitrogen in the rumen resulted in a more efficient synthesis of microbial protein. Possible implications of an improvement in microbial growth include a lower requirement for expensive undegradable protein sources, such as fishmeal (Henning, 1990) and a lower excretion of nitrogen. In order to formulate rations with the S.I.R.E. program raw ingredients, characterised in terms of their rate and extent of organic matter and nitrogen degradability in the rumen are required. The objective of this experiment was to produce a data base of degradability coefficients of various feeds commonly fed to ruminants for

use in the S.I.R.E. program. A sufficient quantity of the feeds characterised were also stored for use in the production studies.

### **3.2. Animals and basal diet**

Four wether sheep aged 2 years, weighing c. 55 kg and fitted with permanent rumen cannulae (37 mm diameter), were housed in individual slatted floor pens with free access to water and mineral licks and kept under continuous lighting. A basal diet containing (g/kgDM) 450 winter wheat straw, 200 barley, 145 sugar beet pulp, 65 white fishmeal, 65 soya bean meal, 50 maize gluten meal and 25 of a mineral vitamin mix was fed in 2 equal portions at 09.00 and 17.00 h at a rate of 1 kg fresh weight per day.

#### **3.2.1. Test feeds and incubation procedure**

The *in situ* degradability of samples of winter barley, citrus pulp, fish meal (batches A and B), malt distillers grains (batches A and B), maize gluten, rape seed meal (batches A and B), sugar beet pulp, soya bean meal, sopralin (formaldehyde treated soya bean meal), sunflower seed extract, tapioca, winter beans and winter wheat straw were determined according to AFRC (1992). The samples were ground through a 2.5 mm screen and material less than 45  $\mu\text{m}$  was removed by hand sieving. Approximately 5.0 g DM of each test feed was accurately weighed into synthetic bags with a pore size of 43  $\mu\text{m}^2$  and thread diameter of 40  $\mu\text{m}$ . The bags were stitched at 40 stitches per inch with polyester thread, turned inside out and re-stitched. In addition stitched areas were sealed with Evostick Colourseal sealant. Bags were held closed with polyester string and to



prevent bags becoming detached in the rumen the string was fastened to bags by wrapping an elastic band around both string and bags. Bags plus sample plus string were weighed and then 6 bags placed into the rumen of each sheep 30 minutes after the morning feed. Bags were retrieved after incubations of 2, 4, 6, 8, 12, 24, 48, 72 and 96 h. After removal from the rumen the bags were washed through the 35 min cold rinse cycle of a domestic washing machine. In addition, synthetic bags containing approximately 5.0 g of dry matter of each feedstuff were washed through the same cold rinse cycle to provide a measure of the immediately soluble fraction. Sufficient bags were inserted into the rumen of each sheep at each time point to provide a pooled residue of approximately 7 g of DM for each incubation time for each sheep. Degradability of each component at each time point was calculated as:

$$1 - \frac{\text{Bag residue (gDM)}}{\text{Feed weighed into bag (gDM)}} \times \frac{\text{Residue concentration of X (g/kgDM)}}{\text{Feed concentration of X (g/kgDM)}}$$

Where X = Nitrogen, Organic Matter

### 3.2.2. CALCULATION OF DEGRADABILITY COEFFICIENTS

Degradability coefficients for the test feeds were calculated by fitting the data obtained for nitrogen and organic matter fractions to the first order model of Ørskov and McDonald (1979) without forcing the zero time points through zero. Degradability coefficients were calculated from the pooled residues:

$$p = a + b(1 - e^{-ct})$$

Where p is the amount of the feed degraded at time t (h), a is the immediately soluble

fraction, b is the insoluble but potentially degradable fraction and c is the constant rate of degradation of the b fraction.

To determine possible lag phases the data were also fitted to the first order model containing a lag phase (Sinclair *et al.*, 1993):

$$\begin{array}{ll} p = a & \text{up to lag (h)} \\ p = a + b(1 - e^{-ct}) & \text{from lag time onwards} \end{array}$$

where a, b, c and lag are as described in the equations above. All degradability calculations were performed using GENSTAT 5 (Lawes Agricultural Trust, 1990).

### 3.3. RESULTS

Proximate analysis of the raw ingredients characterised are presented in Table 3.1. Degradability coefficients for organic matter and nitrogen fractions of the raw ingredients are given in Tables 3.2 and 3.3 respectively.

#### 3.3.1. Organic matter

Treatment of soya bean meal with formaldehyde (sopralin) resulted in a much lower rate (c) of degradation when compared to the untreated soya (soya bean meal = 0.0764, sopralin = 0.0276, Table 3.2). However there did not appear to be any effect of formaldehyde on the immediately soluble fraction, reflected by the similarity of the a fractions of both soya and sopralin (0.084 and 0.074 respectively). Although the rate (c)

and the potentially degradable fraction (b) of the 2 batches of fishmeal were similar, the differences in the immediately soluble fractions (a) resulted in differences in total amount degradable (a + b). The 2 batches of malt distillers dark grains differed in their soluble (a) and potentially degradable (b) coefficients but had similar degradation rates (c). However differences in the immediately soluble (a) and the potentially degradable (b) fractions compensated for each other resulting in the total amount degradable (a + b) being similar for the 2 batches. Feedstuffs high in starch (tapioca, winter beans and winter barley) had comparable and large immediately soluble fractions (a) and both the winter barley and the winter beans had fast rates (c) of degradation (0.2293 and 0.4090 respectively).

### **3.3.2. Nitrogen**

The degradability data for nitrogen were fitted to the first rate model without a lag phase only. The 2 fishmeals varied in their immediately soluble fractions (a) and the potentially degradable fractions (b) the resultant total potentially degradable nitrogen (a+b) was similar (0.9729 and 1.0083). The treatment of soya bean meal with formaldehyde (sopralin) resulted in a lower rate (c) of degradation of nitrogen compared to untreated soya bean meal (0.0158 v. 0.0448) and reduced the soluble fraction (a) from 0.06 to 0.0. Treatment of soya bean meal with formaldehyde resulted in a rate of degradation similar to that of fishmeal. However the total amount of protein that was potentially degradable (a + b) in both soya bean meal and sopralin was similar. The low protein content of winter wheat straw and tapioca made it difficult to fit curves to the nitrogen degradability data and as a result the correlation coefficient ( $r^2$ ) for nitrogen degradability was not

determined for these 2 feeds. Winter beans had the highest soluble nitrogen (a) fraction (0.7907) compared to the other ingredients characterised.

Table 3.1. Dry matter, metabolisable energy content and chemical analysis of 11 protein ingredients.

	Feedstuffs										
	Maize gluten meal	Rapeseed meal (A)	Rapeseed meal (B)	Sunflower seed extract	Winter beans	Soya bean meal	Sopralin	Fishmeal A	Fishmeal B	Malt distillers dark grains (A)	Malt distillers dark grains (B)
Metabolisable energy (MJ/kgDM)	14.4	12.3	12.3	9.9	14.0	13.9	13.5	11.9	13.1	11.2	11.1
Organic matter (g/kgDM)	984.2	913.1	913.1	917.8	956.5	908.1	919.7	784.0	783.5	943.3	941.2
Crude protein (g/kgDM)	646.9	377.5	392.5	298.1	455.6	611.9	527.5	695.0	700.0	268.8	270.6
Acid detergent insoluble nitrogen (g/kgDM)	6.4	7.0	6.2	2.1	0.3	2.6	3.2	0.0	0.0	5.4	1.5
Starch (g/kgDM)	205	10	10	0	319	11	13	0	0	7	15
Water soluble carbohydrate (g/kgDM)	8.9	133.2	134	96.5	90.8	199.2	174.6	0.0	0.0	20.0	23.6
Neutral detergent fibre (g/kgDM)	216.9	477.9	482.0	588.5	230.1	299.0	375.2	0.0	0.0	624.5	454.3
Hemicellulose (g/kgDM)	162.6	192.1	201.1	144.0	82.7	167.6	231.6	0.0	0.0	349.2	306.9
Cellulose (g/kgDM)	35.8	133.7	102.5	258.0	138.4	78.3	64.1	0.0	0.0	141.5	116.7
Ether extract (g/kgDM)	27.6	25.1	22.3	0.8	11.2	13.8	18.7	44.9	64.5	84.8	61.0

Table 3.1 cont. Dry matter, metabolisable energy content and chemical analysis of 6 energy ingredients.

	Feedstuffs					
	Wheat straw	Tapioca	Citrus pulp	Winter barley	Sugar beet pulp	Silage
Metabolisable energy (MJ/kgDM)	6.9	13.0	14.0	12.9	12.1	11.5
Organic matter (g/kgDM)	915.0	941.0	938.2	975.9	932.0	899.3
Crude protein (g/kgDM)	43.8	26.9	68.8	113.1	101.9	172.0
Acid detergent insoluble nitrogen (g/kgDM)	1.4	0.4	0.8	0.2	1.4	0.2
Starch (g/kgDM)	0.0	646.8	0.0	539.8	0.0	0.0
Water soluble carbohydrate (g/kgDM)	14.9	55.6	248.3	36.5	75.3	2.0
Neutral detergent fibre (g/kgDM)	782.7	104.2	336.5	188.0	601.3	476.4
Hemicellulose (g/kgDM)	312.9	32.6	92.1	115.5	332.4	195.5
Cellulose (g/kgDM)	398.9	48.5	132.5	56.7	241.7	260.0
Ether extract (g/kgDM)	10.5	3.8	24.5	15.8	3.1	3.8

Table 3.2. Degradability coefficients of organic matter for 16 raw ingredients. Coefficients are as described in section 3.2.2

	a	b	c	lag	r <sup>2</sup>	a+b
<i>Organic matter</i>						
Maize gluten meal	0.0923	0.8588	0.0496	0.000	0.970	0.9511
Rape seed meal (A)	0.1920	0.5792	0.1071	1.675	0.973	0.7712
Rape seed meal (B)	0.1719	0.5852	0.1024	0.000	0.988	0.7571
Sunflower seed extract	0.2081	0.3588	0.1157	0.000	0.956	0.5669
Winter beans	0.5162	0.3160	0.4090	5.189	0.881	0.8322
Soya bean meal	0.0840	0.8867	0.0764	3.358	0.983	0.9707
Sopralin	0.0742	0.8734	0.0276	0.000	0.972	0.9476
Fishmeal (A)	0.3015	0.5118	0.0161	0.000	0.957	0.8133
Fishmeal (B)	0.4576	0.5424	0.0111	0.000	0.959	1.0000
Malt distillers dark grains (A)	0.3795	0.3625	0.0401	3.290	0.934	0.742
Malt distillers dark grains (B)	0.4875	0.2976	0.0529	0.000	0.850	0.7851
Winter wheat straw	0.0538	0.5083	0.0270	5.280	0.965	0.5621
Tapioca	0.6100	0.2900	0.0984	0.000	0.894	0.9000
Citrus pulp	0.4324	0.5676	0.0876	2.649	0.903	1.0000
Winter barley	0.4768	0.3894	0.2293	0.000	0.882	0.8662
Sugar beet pulp	0.1319	0.7900	0.0545	0.000	0.974	0.9219
s.e.d.	0.06140	0.07070	0.04250	0.7600	-	0.04550

Table 3.3. Degradability coefficients of nitrogen for 16 raw ingredients. Coefficients are as described in section 2.2.3

	a	b	c	r <sup>2</sup>	a+b
<i>Nitrogen</i>					
Maize gluten meal	0.0386	0.9427	0.0385	0.984	0.9813
Rape seed meal (1)	0.1210	0.7690	0.0778	0.956	0.8900
Rape seed meal (2)	0.0924	0.7690	0.1134	0.973	0.8614
Sunflower seed extract	0.3200	0.6053	0.1070	0.951	0.9253
Winter beans	0.7907	0.2185	0.0577	0.577	1.0092
Soya bean meal	0.0600	0.9400	0.0448	0.934	1.0000
Sopralin	0.0000	1.0000	0.0154	0.944	1.0000
Fishmeal (1)	0.2989	0.6740	0.0125	0.982	0.9729
Fishmeal (2)	0.4693	0.5390	0.0131	0.969	1.0083
Malt distillers dark grains (1)	0.5949	0.2997	0.0567	0.712	0.8946
Malt distillers dark grains (2)	0.7436	0.1857	0.0752	0.815	0.9293
Winter wheat straw	0.4652	0.3700	0.0100	nd	0.8352
Tapioca	0.4910	0.0100	0.3764	nd	0.5010
Citrus pulp	0.4281	0.5482	0.0345	0.762	0.9763
Winter barley	0.3721	0.5502	0.1283	0.795	0.9223
Sugar beet pulp	0.0200	0.8300	0.0270	0.945	0.8500
s.e.d.	0.15890	0.10880	0.01783	-	0.12850



### 3.4 Feed assumptions

The chemical analysis of fat prills and urea were obtained from feed tables (AFRC, 1993). Fat prills had values of 950 g/kg, 36.0 MJ/kgDM and 950 g/kgDM for respective content of dry matter, metabolisable energy and ether extract and urea was assigned values of 950 g/kg and 2812.0 g/kgDM for dry matter and crude protein respectively. All other chemical constituents for these two ingredients were assumed to be zero. The immediately soluble organic matter and nitrogen fraction of the feeds were assumed to be degraded in the first hour. Urea was assumed to have an organic matter soluble fraction (a) of 900 g/kgDM and the remaining 100 g/kgDM was degraded at a rate of 50% per h (Sinclair *et al.*, 1995). Minerals and fat were assumed to be undegradable and thus supplied no nitrogen or organic matter to the rumen microbes. The potentially degradable fraction of wheat straw was given a degradation coefficient as used by Sinclair *et al.* (1993).

### 3.5 CALCULATION OF THE HOURLY DEGRADATION

The amount of feed material degraded each hour (P) was calculated using the fractional outflow rate of solids (k) from the rumen as described by Sinclair *et al.* (1993):

$$P = a + ((b \times c)/(c + k)) \times (1 - e^{-(c+k)t})$$

Where t is the time of incubation (h) and a, b, and c are as described in section 3.2.2.

For components that were shown to have a lag phase, degradability was calculated as:

$$P = a \quad \text{up to lag}$$

$$P = a + ((b \times c)/(c + k)) \times (1 - e^{-(c + k) \times (t - \text{lag})}) (e^{-k \times \text{lag}}) \quad \text{from lag time onwards}$$

### 3.6 CALCULATION OF THE SYNCHRONY INDEX

The quantity of organic matter and nitrogen degraded per hour was calculated as the difference between cumulative hours. For example the amount degraded in the 4<sup>th</sup> h of the day was calculated as the total amount degraded over 4 h less the total amount degraded over 3 h. Due to the slow rates of degradation of some of the feeds (Tables 3.2 and 3.3) residues were still present in the rumen after 24 h. To accommodate this the amount of organic matter and nitrogen released in, for example, the 28<sup>th</sup> h after the initial feed, was added to the amount degraded in the 4<sup>th</sup> h and then the total amount degraded in the 27<sup>th</sup> h was subtracted. Hence the amount degraded in the 4<sup>th</sup> h of any 24 h period contained the organic matter and nitrogen released from the 4<sup>th</sup>, 28<sup>th</sup>, 52<sup>nd</sup>, 76<sup>th</sup> h etc after feeding up to a 150 hours.

From the hourly quantity of organic matter and nitrogen degraded in the rumen a synchrony index was calculated from the ratio of grammes of nitrogen and kg organic matter released (Sinclair *et al.*, 1993). The optimum ratio of g nitrogen per kg organic matter released for growing lambs was assumed to be 25 (Czerkawski, 1986). The ratio of g of nitrogen per kg organic matter was calculated for each h of a 24 h period and then a mean value taken. The deviance from the optimum value of 25 was indicated by the

synchrony index where a value of 1.0 represents perfect synchrony whilst values < 1.0 indicate the degree of asynchrony. The synchrony index was calculated from the following equation:

$$\frac{25 - \sum_{1-24} \sqrt{(25 - \text{hourly N/OM})^2}}{25}$$

(Sinclair *et al.*, 1993)

Where 25 = 25 g N/kg organic matter truly digested in the rumen and is assumed to be the optimum ratio of N:OM for rumen microbes in the growing ruminant (Czerkawski, 1986). To enable the synchrony index to be calculated the program requires a total dry matter intake (g/d), the proportion of that total dry matter intake eaten per hour and the predicted outflow rate (per hour) of solids from the rumen.

### **3.6.1 Description of modifications made to the computer program S.I.R.E. (Synchrony Index for the Rumen Environment)**

The computer program S.I.R.E., as described by Sinclair (1992) was used to predict the hourly release of nitrogen and organic matter in the rumen. The chemical analysis (Table 3.1) and degradability coefficients of the raw ingredients (Tables 3.2 and 3.3) were added to the program. The program was modified to predict the supply (g/kgDM) of fermentable metabolisable energy, digestible undegradable protein, effective rumen degradable protein and metabolisable protein as predicted by AFRC (1992).

### 3.7 DISCUSSION

The ingredients characterised represented a wide range in both organic matter and protein degradability characteristics although the composition fell within the range reported by MAFF (1992). Sugar beet pulp had been chosen because this feed would supply a relatively slow source of organic matter to the rumen microbes. Rooke *et al.* (1992) had observed that molassed sugar beet pulp had improved the capture of the immediately available nitrogen associated with grass silage, concluding that the effect was due to the soluble sugars supplied by the molasses. This would make it more difficult to formulate rations based around slow energy degradability for the present work and was therefore the reason for characterising unmolassed sugar beet pulp.

Certain ingredients, namely maize gluten meal, winter beans, soya bean meal and sopralin, had a total chemical composition of over 1000 g/kgDM. This is obviously not possible and is probably due to an over estimation of the carbohydrate fractions associated with these feeds.

Rates and extent of degradability of both N and OM varied widely between individual ingredients as has been observed by others (eg Sniffen *et al.* 1992) and it is clear that different batches of the same feeds exhibited marked differences in degradability characteristics. The OM of the different batches of malt distillers dark grains, rape seed meal and fish meal used in the present experiment not only differed in soluble fractions, potentially degradable fractions, rates of degradation and whether or not they had a lag phase but also varied in proximate analysis compared to other authors who had

characterised different batches of the same ingredients (Sinclair *et al.* 1993; Tamminga *et al.* 1990). The resultant lag phase for the winter beans was probably due to the fibrous nature of the pericarp surrounding the starchy endosperm. Similarly, the small immediately soluble fraction (a) and potentially degradable fraction (b), the slow rate of degradation (c) and the lag phase of winter wheat straw was a reflection of its high NDF content (Table 3.1).

Protein degradabilities varied not only between different batches of the same ingredient in the present work but also in comparison to other authors (see Tables 3.3 and 1.3). The value of fish meal for feeding to ruminants is primarily due to its relatively low protein degradability and appropriate amino acid profile (Chancellor, 1983). It is clear however, that different batches of fishmeal displayed different degradability characteristics and although the total potentially degradable fraction (a+b) of the two fishmeals characterized in the present study were similar, effective rumen degradable protein (and therefore bypass protein) would be considerably different. Implications of this observation are that the potential value of high quality undegradable protein sources will vary from batch to batch. It was therefore important in the current work that the batch of feeds characterised were used in the subsequent production experiments.

The use of fistulated animals and the suspension of synthetic bags in the rumen is not the only method of degradability measurements. Alternative methods currently available include batch culture techniques that measure ammonia production from feeds incubated with rumen liquor (Raab *et al.* 1983), measurement of total gas and pressure production as currently under development at the Institute of Grassland and Environmental Research

(IGER, Automated Pressure Evaluation System) and continuous culture systems to mimic feed intake and outflow to and from the fermenter (Hoover *et al.* 1982). The use of the nylon bag and cannulated animals has a number of advantages over other approaches to feed characterization, namely the variation in microbial synthesis associated with batch culture techniques, the ease for extended incubation times and also the determination of the rate of degradation. There are however a number of areas for potential variation associated with the dacron bag technique, including bag characteristics, sample characteristics, basal diet and animal effect and microbial contamination.

### **3.7.1 Bag characteristics**

The chosen bag porosity is to accommodate two prime functions of influx to the bag of the microorganisms responsible for feed degradation and the efflux of undegraded feed particles (Doreau and Ould-Bah, 1992). In bags with a pore size of 10  $\mu\text{m}$  bacteria presence within the bags is only 10 % of rumen contents but progressively increases as pore size increases (Meyer and Mackie, 1983) and a more diverse microbial colonization of feed samples was observed by Varvikko and Lindberg (1985) when the use of 40  $\mu\text{m}$  bags were compared to 20  $\mu\text{m}$  bags. In addition it has also been suggested that a pore size below 10  $\mu\text{m}$  restricts the entry of protozoa into the bags which could ultimately affect the degradability coefficients of feed samples (Meyer and Mackie, 1983). Reducing porosity may also inhibit the removal of degradation end products (causing end product inhibition to microbes), inefficient washing procedure, which may underestimate the immediately soluble and slowly degradable fractions and possibly prevent the removal of rumen associated material after retrieval of the bags from the rumen, and also

entrapment of gasses, which could also inhibit the actions of the hydrolytic microorganisms. Weakley *et al.* (1983) observed decreases in both nitrogen and dry matter degradability values irrespective of incubation length when pore size was reduced from 53  $\mu\text{m}$  to 5  $\mu\text{m}$ . However the decrease was an absolute value and the ratio of nitrogen to dry matter degraded remained relatively unaffected.

Increasing pore size will increase the undegraded particle loss which would then be assumed as part of the degraded fraction. However this would only cause an over estimation of the extent of feed degradability if the escaped particles were degraded at a slower rate than the retained particles. AFRC (1992) recommended a pore size of between 40 - 50  $\mu\text{m}$  and the bags used in the present study had a pore size of 42  $\mu\text{m}$ .

### **3.7.2 Sample characteristics**

Presentation of the sample within the dacron bags attempts to mimic the effects of mastication by the host. Generally feeds are ground although the evidence of the influence of particle size on the rate and extent of sample degradation is contradictory. Freer and Dovi (1984) ground lupins through a selection of different screens, 0.8 mm (fine), 4.0 mm (medium) and unground (coarse) to assess the effect of grinding on degradability. Although the total potentially degradable fractions (a+b) were similar, the immediately soluble fraction (a) ranged from 0 to 74.3 % and the potentially degradable fraction (b) from 100 to 21.9 % for coarse to fine ground respectively. The rates of degradation were six times faster for fine ground compared with coarse material. Additionally, disappearance of dry matter was less affected than disappearance of

nitrogen in the various ground samples. Similar results were observed by Weakley *et al.* (1983) but conversely, Ehle *et al.* (1982) concluded that particle size had no significant effects on dry matter and nitrogen kinetics. AFRC (1992) recommended a feed particle size of between 45  $\mu\text{m}$  and 2.5 mm and samples were processed in the present experiment by grinding through a 2.5 mm mill. In an attempt to minimise any possible differences in the ratio of release of energy and nitrogen yielding substrates in the subsequent growth trials, rations were presented to the animals at a similar particle size as was used during the characterization process.

The size of the sample introduced into the bags may also affect the degradability (Lindberg, 1981). Mehrez and Ørskov (1977) noted that decreasing the ratio of sample size to bag surface area from 55 to 15  $\text{mg}/\text{cm}^2$  increased the dry matter disappearance from 37.5% to 85% after a 24 h incubation. As the amount of food introduced into the bag is increased the more likely is the formation of a tight bolus and a consequential decrease in exposed sample surface area. This ultimately decreases the rate of degradation and could possibly introduce a lag phase. For continuity during the present study a constant 5 g dry matter was weighed into the bags as recommended by AFRC (1992).

### **3.7.3 Basal diet and animal effects**

Microbial colonization of the fermenter is greatly dependent on the basal diet offered (Leng and Nolan, 1984) and may affect the extent and rate of degradation of test samples. Table 1.2 pointed out a number of factors that could alter the degradability characteristics



of fibre, several of which are directly influenced by the basal diet offered. An example of such is indicated by the work reported by Weakley *et al.* (1983) who found lower dry matter and protein degradabilities from ruminants fed high, compared to low, concentrate:forage diets. Similarly, Lindberg (1981) found a significant effect of basal diet on protein and dry matter disappearance from dacron bags. Weakley *et al.* (1983) suggested that large amounts of bacterial slime associated with maize diets may block the pores of the bags preventing escape or access of microorganisms. Further work is required in this area to assess the effects of bacterial slime on microbial degradation. However neither Weakley *et al.* (1983) nor Lindberg (1981) observed differences in the ratio of dry matter to nitrogen degraded in the test feeds incubated.

The basal ration used for the present experiment was formulated using a mixture of the raw ingredients due to be characterised (section 3.2). The basal ration satisfied the requirements of rumen microbes for nitrogen, indicated by the predicted effective rumen degradable protein to fermentable metabolisable energy ratio (10.3 g ERDP/MJFME) and therefore it was accepted that the degradation of the test feeds would not be limited by low rumen ammonia, an important factor that may affect the degradability coefficients (Mehrez and Ørskov, 1977). The level of feeding of the basal diet remained constant (1 kg fresh feed per day) throughout the degradation study for all the feeds characterized and therefore each sample evaluated was subject to the same rumen environment.

Huntington and Givens (1995), in their review of sources of variation encountered using dacron bags to estimate degradability, split the effects of the host into three areas, namely inter-species, intra-species and inter- and intra-animal. The inter-species variation did not

apply to the present study as feed characterization and subsequent experiments based on the results of the characterization period were all undertaken using sheep. Physiological status of the animals used for characterization and the growth experiments were different as the wether sheep used to determine the degradability characteristics were fed at approximately 1.1 times maintenance (energy requirements). It could be argued that the level of feeding should have been more representative of the subsequent experiments during the characterization stage. To avoid an absolute value of degradation of feed material in the rumen the first order model used to estimate the degradability coefficients of raw ingredients (Ørskov and McDonald, 1979) produces values that, when used in conjunction with rumen outflow rate are dynamic and provide effective degradability figures.

#### **3.7.4 Microbial contamination**

Microbial contamination of feed residues has the potential to alter the estimated degradability coefficients (in particular nitrogen) and cause significant errors or variation especially in low protein fibrous ingredients such as straw (Nocek, 1985). Washing the bags after rumen incubation removes much of the rumen liquid but can still leave significant quantities of microbial matter (Doreau and Ould-Bah, 1992). This bacterial colonization leads to an under estimation of ruminal degradability of dietary nitrogen and will therefore tend to lower the ratio of nitrogen to organic matter degraded. The extent to which this ratio may vary is dependent upon the feed being examined. It has been observed that the variability of protein degradation ranged from 71 degradability points for straw to zero for soyabean meal (Doreau and Ould-Bah, 1992). Doreau and Ould-Bah

(1992) estimated that feeds with a 2 % nitrogen content would have an under estimate of 10 degradability units which approaches zero error at a nitrogen content of approximately 8 %. Further evidence for this reduction in variability is presented in Table 3.4, where the percentage error in nitrogen degradability for various feeds of different nitrogen content is estimated.

Table 3.4. Error (%) in estimates of nitrogen degradability caused by microbial contamination of feed residues.

Feed	†Crude protein content (g/kgDM)	Incubation time (h)		
		5	12	24
		Percentage error		
Rape seed meal	378	4.4	1.2	0.7
Barley	113	26.1	10.5	3.9
Ryegrass	90	13.2	6.4	5.5
Straw	44	221	310	1285

Adapted from Huntington and Givens (1995)

†Estimated values

Table 3.4 indicates the importance of microbial contamination for slow degrading, low protein feedstuffs. The feeds used in the present experiment varied in their nitrogen content from 7 g/kgDM (straw) to 125 g/kgDM (fishmeal). The feeds chosen as protein sources all had relatively high protein contents and would therefore have been minimally affected by colonization. The energy feeds used were more likely to have produced under estimates of nitrogen degradability but, because of the relatively low nitrogen content of these feeds (eg straw, sugar beet pulp and tapioca) they would not have had a major influence on the ratio of N:OM released once part of the total ration.

It is clear that the degradability of organic matter and nitrogen can be affected by various conditions during the incubation procedure. However the majority of the effects appear to result in alterations in the absolute amounts of nitrogen and organic matter degraded with less of an effect on their ratio of release.

### **3.8 CONCLUSIONS**

Different batches of the same feed displayed variation in their degradability characteristics.

Samples characterised in the present experiment differed in their degradability data compared to other authors who had characterised different batches of the same feed.

## **CHAPTER 4**

### **4.0 EFFECTS OF SYNCHRONISING THE HOURLY RELEASE OF ENERGY AND NITROGEN IN THE RUMEN IN DIETS WITH TWO CARBOHYDRATE SOURCES ON THE PERFORMANCE OF GROWING LAMBS**

#### **4.1 Introduction**

The formulation of diets that are synchronous for their hourly release of energy and nitrogen in the rumen has been seen to promote a greater efficiency of microbial protein production (Sinclair *et al.*, 1993; Sinclair *et al.*, 1995). Despite this suggestion little work has been completed to evaluate the effects of dietary synchrony on host animal performance. The objectives of the following experiments were :

1. to use the modified SIRE computer program and the degradability data collected described in chapter 3 to formulate diets to be either synchronous or asynchronous with respect to their hourly release of energy and nitrogen in the rumen,
2. to evaluate the diets described in objective 1 on aspects of rumen and whole body metabolism, and growth and carcass characteristics in growing male lambs fed at a restricted level of intake,
3. to evaluate the diets described in objective 1 on aspects of whole body

and rumen metabolism and growth and carcass characteristics in ewe lambs fed *ad libitum*.

#### 4.2 Formulation of experimental diets

Diets (Table 4.1) were formulated using the S.I.R.E. computer program (section 3.5.1) to differ in their rate and extent of hourly organic matter and nitrogen release in the rumen, as indicated by the synchrony index (section 3.5). Diets were designed to have similar contents of metabolisable energy (10.4 MJ/kgDM), digestible undegradable protein (32 g/kgDM) and metabolisable protein (86 g/kgDM) (Table 4.1). Four diets were formulated, two to have a high degree of rumen synchrony and two to be less synchronous. The formulations assumed a dry matter intake of 1000 g per 24 h fed in 2 equal portions, one in the 1<sup>st</sup> hour and the second 8 hours later. A rumen outflow rate of 0.046/h was chosen based on the level of feeding which at 150 g of daily live weight gain is equivalent to 1.76 times maintenance. (AFRC, 1992).

$$r = -0.024 + (0.179 \times (1 - e^{-0.278L}))$$

Where L = level of feeding and r is the rumen outflow rate.

The diets were slow hourly release of energy, synchronous (SS) deemed to have a synchrony index of 0.84, a slow release of energy, asynchronous (SA) with a synchrony index of 0.54, a fast release of energy, synchronous (FS) having a synchrony index of 0.87 and a fast release of energy, asynchronous (FA) with a synchrony index of 0.51.

Table 4.1. Synchrony indices, dietary composition and predicted content of metabolisable energy, crude protein, fermentable metabolisable energy, digestible undegradable protein, metabolisable protein, neutral detergent fibre and starch content of four diets. Rumen outflow rate is 0.046/h.

	Diets			
	SS <sup>a</sup>	SA <sup>b</sup>	FS <sup>c</sup>	FA <sup>d</sup>
<sup>†</sup> Synchrony index	0.84	0.54	0.87	0.51
Dietary composition (g/kgDM)				
Wheat straw	292.5	300	340	350
Barley	135	--	480	--
Tapioca	--	--	20	472
Sugar beet pulp	400	470	20	--
Winter beans	--	100	80	80
Fishmeal	--	--	--	58
Sopralin	--	--	30	--
Malt distillers dark grains	--	100	--	--
Sunflower seed extract	67.5	--	--	--
Rape seed meal	60	--	--	--
Urea	10	5	5	15
Fat	10	--	--	--
Mineral/vitamins	25	25	25	25
ME (MJ/kgDM)	10.4	10.3	10.6	10.5
Crude protein (g/kgDM)	140	148	138	147
FME (MJ/kgDM)	9.7	10.2	10.0	9.4
Metabolisable protein (g/kgDM)	87	88	84	84
DUP (g/kgDM)	37	35	28	26
NDF (g/kgDM)	538	567	416	354
Starch (g/kgDM)	73	33	297	331

<sup>a</sup>slow energy, synchronous, <sup>b</sup>slow energy, asynchronous, <sup>c</sup>fast energy, synchronous, <sup>d</sup>fast energy, asynchronous.

<sup>†</sup>Calculated as described in section 3.5

The raw ingredients for the above diets (Table 4.1) were chosen primarily on their degradability characteristics of organic matter and nitrogen (Tables 3.2 and 3.3). For comparative purposes the diets were also formulated with the intention of keeping the



supply of metabolisable energy and protein to the host similar per kgDM fed (Table 4.1). All diets contained approximately 30-35% winter wheat straw and 2.5% of a mineral supplement (Intensive Lamb, Trouw Nutrition). Although the predicted crude protein content of the FS diet was approximately 5% lower than the mean crude protein content of the other diets, the predicted supply of metabolisable protein was comparable between the four mixes (Table 4.1). Diets supplying either a fast or slow release of energy had a similar predicted level of plant cell wall material and starch respectively. (Table 4.1).

#### **4.2.1 Level of feeding for animals fed at a restricted level of intake**

The level of feeding chosen for animals described in objective 2 above was sufficient to supply enough metabolisable energy for 150 g of live weight gain per day (AFRC, 1992), which equates to a level of feeding of 1.75 times maintenance (energy basis). This growth rate was chosen so that lambs were capable of a constant live weight gain as animals approached their mature weight (approximately 50 kg). The predicted supply of metabolisable protein was calculated to be deficient for lambs below c. 34 kg live weight and excessive for lambs above this live weight (Fig. 4.1; calculated from the prediction equations of AFRC, 1992).

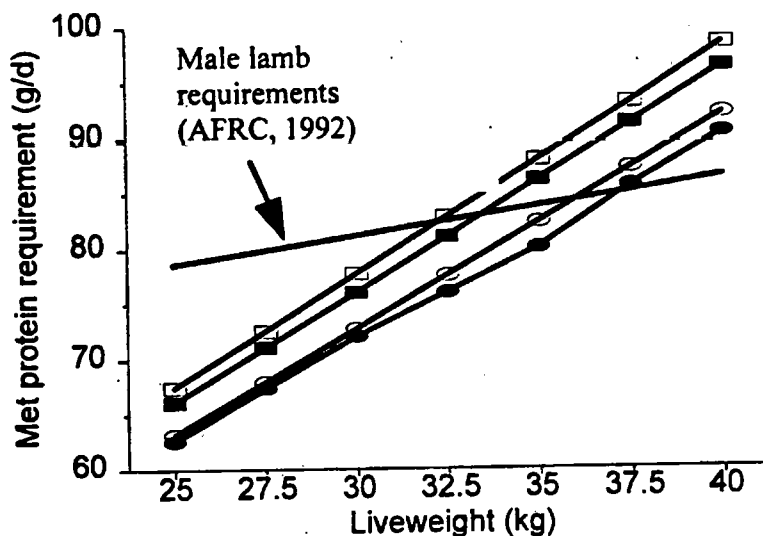


Fig. 4.1. Requirements for metabolisable protein of male lambs growing at 150 g per day calculated as AFRC (1992) and the predicted supply of metabolisable protein for diets formulated to have a slow rumen hourly release of energy and synchronous (■), a slow release of energy and asynchronous (□), a fast release of energy and synchronous (●) and a fast release of energy and asynchronous (○) in the rumen.

The extent of degradability of organic matter and nitrogen on a daily basis and the predicted release of organic matter and nitrogen for each h over a 24 h period are presented in Table 4.2 and Fig. 4.2 respectively.

Table 4.2. Predicted daily extent of organic matter and nitrogen degraded in the rumen of 4 diets differing in their pattern of energy and nitrogen release in the rumen.

	Diet			
	SS <sup>a</sup>	SA <sup>b</sup>	FS <sup>c</sup>	FA <sup>d</sup>
Organic matter degraded (g/d)	439	426	536	520
Nitrogen degraded (g/d)	14.0	16.0	16.0	18.0
Organic matter degradability (gOM/gOM)	0.49	0.47	0.58	0.58
Nitrogen degradability (gN/gN)	0.63	0.67	0.74	0.77
Daily ratio of N:OM degraded in the rumen	32	37	31	35

<sup>a</sup>slow energy, synchronous, <sup>b</sup>slow energy, asynchronous, <sup>c</sup>fast energy, synchronous, <sup>d</sup>fast energy, asynchronous.

Calculations are based on 1000 g dry matter fed in two equal meals 8 h apart and a rumen outflow rate of 0.046/h.

The predicted amount of organic matter degraded over a 24 h period (Table 4.2) is reflective of the energy sources supplied by the four diets. The two slower release diets primarily supply plant cell wall material with the fast release formulations supplying principally starch (Table 4.1). As a consequence, the diets containing faster release sources of energy were predicted to have higher amounts of organic matter degraded (Table 4.2). The daily nitrogen degraded and the overall daily ratio (g nitrogen degraded/kg organic matter) degraded are similar for all four diets and above the optimum of 25 gN/kgOMTDR (Czerwaski, 1986). The similarity in predicted levels of metabolisable energy, metabolisable protein (Table 4.1) and the daily ratio of g nitrogen degraded per kg organic matter degraded (Table 4.2) indicates that the four diets formulated would promote similar levels of productivity in growing lambs according to current ration formulation (AFRC, 1993). The difference between the diets is principally in their pattern of hourly release of nutrients in the rumen (Fig 4.2).

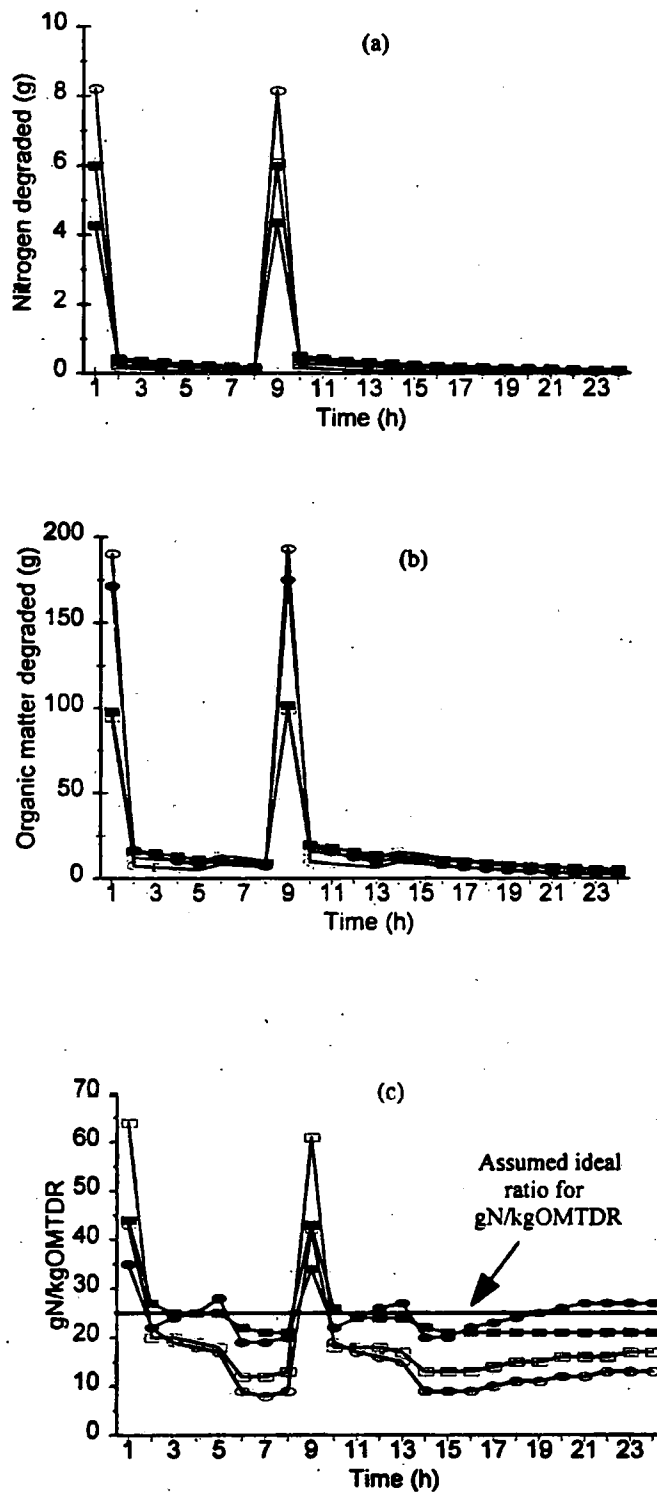


Fig. 4.2. Predicted rates of release of (a) nitrogen and (b) organic matter and (c) predicted ratio of g nitrogen degraded per kg of organic matter degraded in the rumen for diets formulated to have a slow hourly release of energy and synchronous (■), a slow release of energy and asynchronous (□), a fast release of energy and synchronous (●) and a fast release of energy and asynchronous (○). Calculations are based on a total of 1000 g of dry matter fed in two equal meals, the first meal in the first hour, the second in the ninth hour and a rumen outflow rate of 0.046/h.

Predicted peaks of nitrogen supply for the asynchronous diets tended to be higher than the two synchronous diets (Fig 4.2a). The amount of organic matter degraded in the first hour reflects the choice of energy contributing ingredient of that diet (Fig 4.2b). Diets based on starch (FS and FA; Table 4.1) had an additional 84.7 g of organic matter degraded in the first hour compared to the diets based on digestible fibre (SS and SA; Table 4.1). The hourly supply of organic matter from diets containing the quick release of energy (FS and FA) became lower from the third hour after feeding compared to diets SS and SA (Fig 4.2).

#### **4.3 RESULTS**

The chemical analyses of the 4 diets is presented in Table 4.3. The organic matter content (g/kgDM) of the four experimental diets was similar. Although the amount of crude protein was approximately 10 g/kgDM lower than predicted values for diets SA, FS and FA protein contents were similar for diets SS, SA and FA ( $138 \text{ g/kgDM} \pm 1.25$ ) but lower for diet FS ( $124 \text{ g/kgDM}$ ). The content of NDF present in the four diets was close to the predicted values (Table 4.1). Diets formulated to have a slow energy release (SS and SA) were higher in their NDF content compared to diets supporting a quick energy release (FS and FA).

Table 4.3. Chemical analysis (g/kgDM) of diets formulated to differ in the rate and extent of energy and protein degradation in the rumen.

	Diets			
	SS <sup>a</sup>	SA <sup>b</sup>	FS <sup>c</sup>	FA <sup>d</sup>
Organic matter	914	919	925	900
Crude protein	138	139	124	136
Water soluble carbohydrate	75	68	43	28
Starch	66	32	260	239
Neutral detergent fibre	549	569	419	350
Hemicellulose	267	299	213	167
Cellulose	235	233	164	141
Ether extract	17	15	16	13

<sup>a</sup>slow energy, synchronous, <sup>b</sup>slow energy, asynchronous, <sup>c</sup>fast energy, synchronous, <sup>d</sup>fast energy, asynchronous.

## 4.4 DISCUSSION

### 4.4.1 Choice of ingredients

The formulation of the diets for the present experiments were achieved by varying the ingredient composition. Using this approach to vary the rate and extent of rumen availability of energy and protein yielding substrates has been criticised (Chamberlain and Choung, 1995) as effects of different ingredients may confound results. For example, the different ingredients would supply different individual carbohydrates, peptides and amino acids, all important factors involved with efficient microbial growth. The work completed recently by Henderson *et al.* (1998) chose to alter the degree of synchrony by feeding the same ingredients at different times of the day to avoid this problem. However this approach may lead to detrimental rumen environments (eg rapid lowering of pH caused by a sudden intake of rapidly degradable starch) which may be detrimental to microbial growth. The various ingredients were chosen for the rations in the present trial not only to provide different synchrony indices (Sinclair *et al.* 1993) but also provide similar predicted metabolisable nutrients to the host (AFRC, 1992). The resultant formulations (Table 4.1) were not biased toward synchrony. Indeed, diets promoting asynchrony (SA and FA) had the highest crude protein content and contained fish meal, an excellent source of undegradable amino acids that has been demonstrated to promote both microbial and animal growth (Chancellor, 1983).

The different microbial efficiencies observed by Chamberlain *et al.* (1993) when supplying various carbohydrates is an indication that ingredient effects exist, not only in

their utilization for growth but also in the rumen population they sustain (Salter *et al.* 1983). However current ingredient analysis and characterisation is unable to quantify types of individual sugars, amino acids, quantity of peptides, types of fatty acid, bioavailability of minerals and vitamins etc. Additionally, it is difficult to differentiate from the work of Chamberlain *et al.* (1993) whether it was the availability of some undetermined nutrient or its rate of release that was primarily responsible for the variation in microbial efficiency.

The mechanistic approach to diet formulation undertaken by the present work would tend to suggest that other authors (eg Casper and Schingoethe, 1989) may not be achieving particularly large differences in supposedly synchronous and asynchronous formulations through their choice of ingredients. In addition much work investigating varying rates of energy and nitrogen release in the rumen has relied heavily on urea as the nitrogen source (Newbold and Rust, 1992; Henning *et al.*, 1993; Hussein *et al.*, 1991; Rooke *et al.*, 1987; Mansfield *et al.*, 1994) particularly for an asynchronous type diet. Urea, due to its very rapid rate of solubilization, lack of carbon skeletons and no sulphur content is known to reduce the efficiency of microbial synthesis (AFRC, 1984) possibly confounding observed results. The release of nitrogen relative to organic matter in the present study was predicted using the computer program validated by Sinclair *et al.* (1993) and was therefore possible to monitor the predicted release of nutrients (Fig 4.2) making it more likely to achieve the required differences. Additionally, although urea was present in diets it only ranged from 0.5 to 1.5 % inclusion rate and because other proteinaceous material was present, the supply of true protein and therefore amino acids and peptides needed for efficient microbial growth (Sehgal and Makkan, 1994) was probably met.



It was difficult to formulate a ration that had a fast rate of energy but a slow rumen release of nitrogen, without drastically over supplying digestible undegradable protein. Slowly degradable protein sources such as fish meal and sopralin have high proportions of bypass protein at relatively slow rumen outflow rates thus limiting their use in these experiments. Diet FA was based around tapioca (fast energy) and fish meal (slow nitrogen). However, to ensure similar levels of effective rumen degradable protein as the other diets it was necessary to include some urea and winter beans, both good sources of fast rumen degradable nitrogen.

#### **4.4.2 Level of feeding**

The diets were formulated using a rumen outflow rate of 0.046 which relates to a level of feeding of 1.75 times maintenance (energy basis), sufficient for approximately 150 g of daily live weight gain (AFRC, 1992). The ram lambs used during the experiment (Friesland X Charolais) were considered to be capable of growth rates of 200-250 g/d (Notter *et al.*, 1991). However as animals approach their mature weight it becomes increasingly difficult for them to consume sufficient feed to maintain a high level of growth. Therefore to enable a constant rate of gain through out the experiment animals were fed to promote a lower gain.

The pattern of live weight gain has been described as following a sigmoid growth curve, not only for total live weight but also for individual body tissues. Live weight gains of animals fed balanced rations at low body weight relative to mature weight consists of higher proportions of lean compared to lipid but is continuously reduced as animals

approach and reach their mature weight. This is reflected in the requirement for metabolisable energy and protein as the animals grow (Fig 4.3). The ratio of metabolisable protein to metabolisable energy (MetP:MetE) gets progressively less (AFRC, 1992), due to the genetic potential for an increase in lean mass being reached and energy required for fat deposition and maintenance progressively increasing.

A similar picture can be observed in the rumen. A young animal has a restricted rumen in terms of size and therefore the microbial protein output cannot sustain the young growing animal. Fig 4.4 shows the proportion of metabolisable protein supplied by microbial protein needed for a male lamb growing at 150 g/d. As live weight increases the proportion of metabolisable protein supplied by microbial protein also increases (Fig 4.4).

A more synchronous release of energy and protein in the rumen has been reported by different authors to increase the quantity and efficiency of microbial output (Sinclair *et al.*, 1993,1995; Aldrich *et al.*, 1993; Herrera-Saldana, 1990; Hussein *et al.*, 1991) and an objective of the current experiments was to measure any effects of synchrony on host growth characteristics. A young animal will respond to protein supplementation (increased supply of digestible amino acids at the small intestine) at a lighter live weight as illustrated by Figs 4.3 and 4.4. For this reason the diets were formulated to be deficient in protein supply at a live weight below 34 kg in order to allow any effects of increased microbial protein supply to be more fully realised.

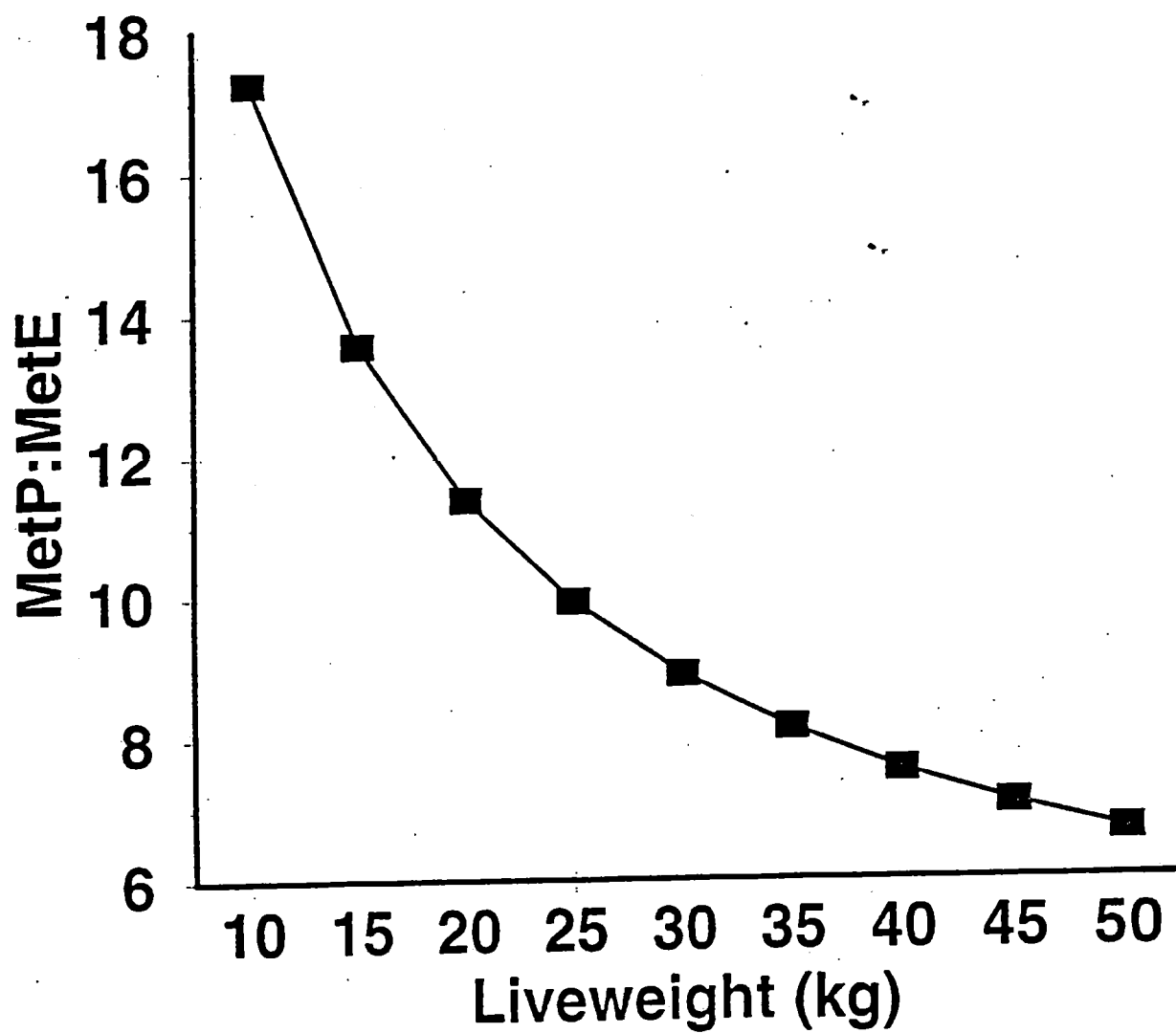


Fig 4.3. The changing ratio of metabolisable protein to metabolisable energy of male lambs growing at 150 g/d. (Calculated as AFRC, 1992).

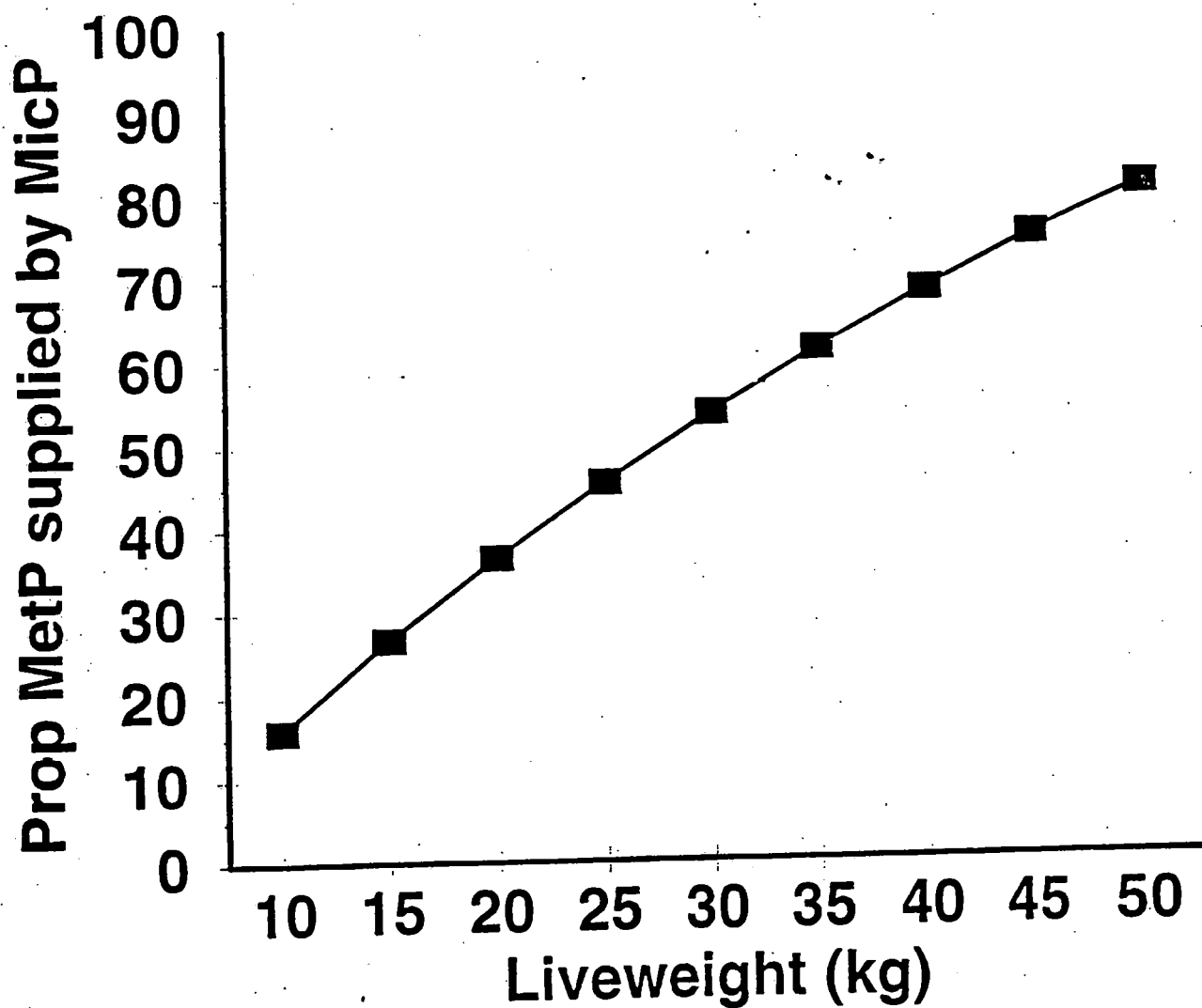


Fig 4.4. The proportion of metabolisable protein supplied as microbial protein in male lambs growing at 150 g/d. (Calculated as AFRC, 1992).

## **4.5 THE EFFECTS OF SYNCHRONIZING THE RATE OF ENERGY AND NITROGEN SUPPLY IN THE RUMEN IN DIETS WITH TWO CARBOHYDRATE SOURCES ON METABOLISM IN MALE LAMBS**

### **4.5.1 Introduction**

The increased efficiency of microbial protein synthesis observed by Sinclair *et al.* (1993) and Sinclair *et al.* (1995) when feeding diets that were synchronous in their hourly release of energy and nitrogen in the rumen may also result in enhanced production performance. Possible mechanisms for this include reduced nitrogen excretion in urine due to reduced plasma urea-N levels, reflective of periods of nitrogen excess in the rumen. An implication of a more efficient nitrogen capture is more protein available for (a) microbial growth and/or (b) host animal protein requirements. A reduction in nitrogen excretion would also have benefits to the environment. Similarly a synchronous rate of supply of nutrients to the microbial biomass may reduce the incidence of energy spilling reactions during periods of energy excess, resulting in a more efficient fermentation of organic matter per unit of substrate, which may also affect cell composition of rumen microbes (Nocek and Russell, 1988).

The following experiment aimed to investigate the effects of the diets described in section 4.2 on microbial protein synthesis and on whole body metabolism in growing male lambs fed at a restricted level of intake.

#### **4.5.2 Animals and experimental procedure**

Sixteen 10 week old Charolais X Friesland male lambs weighing c. 30 kg were blocked according to live weight and then randomly allocated to one of the four dietary treatments. After an adaptation period of 9 days animals were moved to metabolism crates and subjected to a further 5 days adaptation. Animals were kept under continuous lighting with free access to water at all times. All animals were tethered by the neck to prevent them turning round whilst in the metabolism crates.

#### **4.5.3 Experimental diets**

Diets (section 4.2) were offered at a restricted level of feeding (1.76 times maintenance, energy basis) in two equal portions at 0900 and 1700 h. All individual ingredients were fed in a meal form and were ground through a 2 mm screen prior to mixing to provide a similar physical form to the ingredients incubated in the synthetic fibre bags.

#### **4.5.4 Collection and analysis**

##### **4.5.4.1 Feeds**

Samples (100 g) of the diets for the three experiments described in chapter 4 were collected fortnightly, dried to a constant weight, pooled and then analysed for organic matter, crude protein, neutral detergent fibre, acid detergent fibre, acid detergent lignin and ether extract (section 2.1).

#### **4.5.4.2 Urine collection**

Total urine output was collected daily for 7 days into sulphuric acid (1 M, 100 ml/d) to give a final pH of less than 3. Total volume of acidified urine was recorded and then filtered through glass wool. Sub-samples (20 ml) of the filtrate were taken and then diluted 1:5 with distilled water. Samples were then frozen at -20°C prior to subsequent analysis.

#### **4.5.4.3 Faecal collection**

Total faeces was collected for 7 days using faecal harnesses. The total amount of faeces was weighed and then sub-sampled (5%). All samples were stored at -20°C prior to subsequent analysis.

#### **4.5.4.4 Blood collection**

On day 8 of the collection period the necks of the lambs were sheared and a local anaesthetic cream (Emla cream (5%), Astra Pharmaceuticals, Kings Langley, England) applied. Indwelling jugular catheters (Portex I/V cannula, Sims medical distributors, West Midlands) were inserted into each sheep and their necks bandaged. The catheters were flushed daily and between each sampling with heparinised saline solution. On day 9 hourly blood samples (7 ml) were taken from each lamb, beginning 30 mins before the morning feed, into heparin and potassium oxalate tubes (vacutainer) for a 24 h period. Blood samples were immediately centrifuged (500 g) and the plasma removed and stored

at -30°C prior to subsequent analysis.

#### **4.5.4.5 Urine analysis**

Urine samples (1 ml) were added to digestion tubes containing 14 ml of concentrated sulphuric acid and heated at 450°C for 45 minutes. Samples were then diluted by adding 75 ml of deionised water and then analysed for nitrogen (section 2.1.4) content. Intestinal flow of microbial nitrogen was estimated based on the urinary excretion of purine derivatives according to the method of Chen *et al.* (1990) for uric acid, xanthine and hypoxanthine and Chen *et al.* (1993) for allantoin. The daily output of purine derivatives in urine was calculated and then used to estimate the amount of microbial nitrogen as described by Chen *et al.* (1992).

#### **4.5.4.6 Faecal analysis**

Daily samples were dried in an oven at 60°C until a constant weight was achieved. Samples were then pooled, ground through a 1 mm screen and analysed for organic matter (section 2.1.2) and nitrogen (section 2.1.4) content.

#### **4.5.4.7 Blood analysis**

Plasma samples were thawed at room temperature and those preserved with potassium oxalate were analysed for  $\beta$ -hydroxy butyrate (Sigma test kit: Randox Laboratories, cat. No. RB532) whilst those preserved with heparin were analysed for urea (Boehringer



Mannheim GmbH, cat. No. 620408) using a Bayer Technicon RA-1000 autoanalyzer.

#### **4.5.4.8 Statistical analysis**

All results were subjected to a randomised block analysis of variance in a 2 X 2 factorial arrangement. Treatment source was sub-divided into energy release, degree of rumen synchrony and interaction. All statistical analysis was performed using GENSTAT 5.1 (Lawes Agricultural Trust, 1990).

### **4.6 RESULTS**

Organic matter digestibility, N intake, faecal N excretion, urinary N excretion, microbial N production, N retention, daily output of purine derivatives, plasma urea-N and plasma  $\beta$ -hydroxy butyrate concentrations in sheep fed the four diets are presented in Table 4.4. There was no significant difference in whole tract organic matter digestibility or N retention (g/g intake) in animals fed any of the diets, however an asynchronous supply of energy and nitrogen significantly increased the total N retained ( $p < 0.05$ ). There was a tendency for diets that were synchronous (SS and FS v. SA and FA) to have a greater organic matter digestibility than those with an asynchronous release of nutrients (Table 4.4) although this was not significant. Animals receiving diet FS had a significantly ( $p < 0.05$ ) higher production of microbial N than animals fed diets SS or FA (Table 4.4). There was a significant interaction between energy and degree of synchrony with animals fed diet FS having a significantly enhanced production of microbial-N and allantoin compared to animals fed diet FA ( $p < 0.05$ , Table 4.4). Mean plasma metabolite

concentrations (urea and BHB mmol/l) were not significantly altered by dietary treatment.

Table 4.4. Mean organic matter digestibility (g/g intake), feed N (g/d), faecal N (g/d), urine N (g/d), Microbial N (g/d), retained N (g/d and g/g), purine derivative (PD) excretion (mmol/d), microbial N production (g/d), plasma urea (mmol/l) and plasma  $\beta$ -hydroxybutyrate (mmol/l) of 4 diets fed to male lambs.

	Diets*		Significance of main effects					
	SS	SA	FS	FA	s.e.d.	Energy	Synchrony	Interaction
Organic matter digestibility	0.654	0.647	0.674	0.667	0.0134	NS	NS	NS
Feed N	18.84	19.25	17.07	18.37	0.463	**	*	NS
Faeces N	5.91	6.20	5.48	5.17	0.371	*	NS	NS
Urine N	7.34	6.93	6.48	6.74	0.444	NS	NS	NS
Microbial N	5.15	6.15	7.80	4.84	1.154	NS	NS	*
Retained N (g/d)	5.59	6.12	5.11	6.47	0.556	NS	*	NS
Retained N (g/g)	0.297	0.317	0.300	0.352	0.0294	NS	NS	NS
PD excretion								
Allantoin	4.83	5.62	7.82	4.71	1.196	NS	NS	*
Uric acid	1.310	1.565	1.125	1.143	0.2491	NS	NS	NS
X + H <sup>†</sup>	0.130	0.155	0.255	0.115	0.0899	NS	NS	NS
Sum of purine derivatives	6.28	7.34	9.20	5.97	1.387	NS	NS	NS
Plasma urea	5.04	4.40	4.30	5.58	0.230	NS	NS	NS
Plasma β-hydroxy butyrate	0.525	0.535	0.545	0.755	0.1286	NS	NS	NS

<sup>\*</sup>SS = Slow release of energy and synchronous, SA = slow release of energy and asynchronous, FS = fast release of energy and synchronous, FA = fast release of energy and asynchronous.

<sup>†</sup>X + H = Xanthine plus hypoxanthine.

NS = not significant; \* =  $p < 0.05$ , residual degrees of freedom = 9.

Diets were offered in two equal meals at 09.00 and 17.00 h.

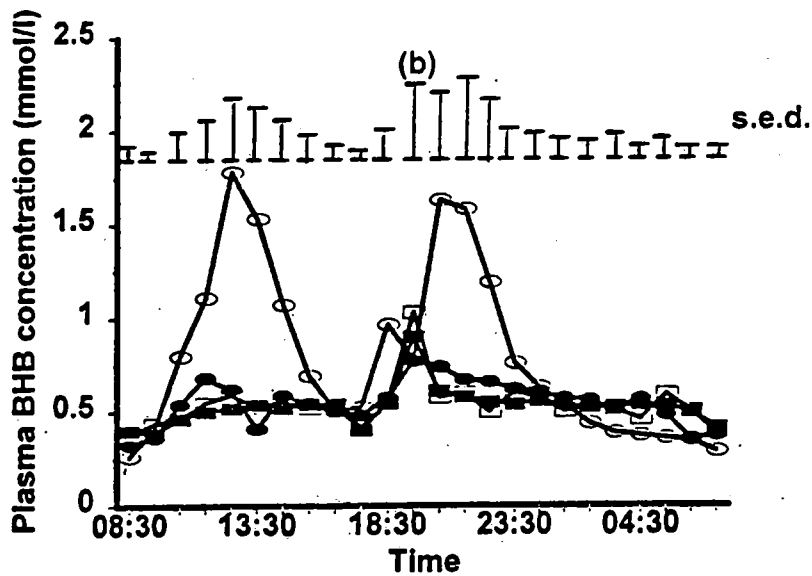
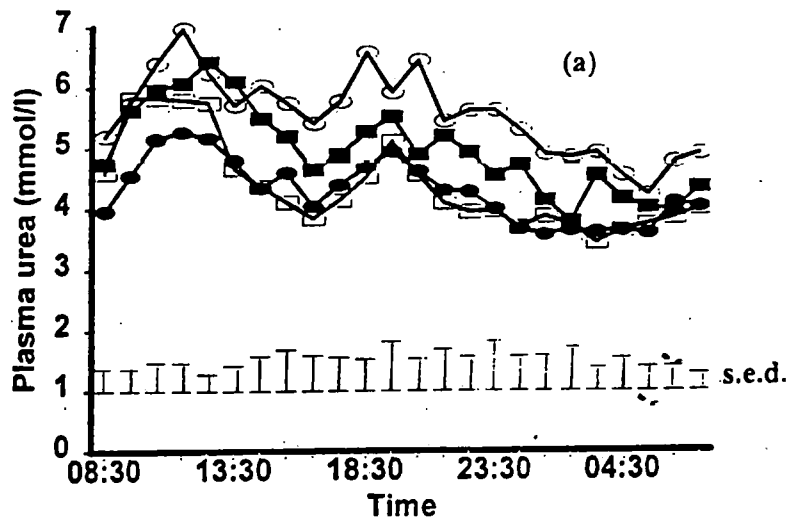


Fig 4.5. Mean plasma concentrations of (a) urea (mmol/l) and (b)  $\beta$ -hydroxybutyrate (mmol/l) of male lambs fed diets formulated to have either a slow release of energy and asynchronous (■), slow energy and asynchronous (□), fast energy and synchronous (●) or fast energy and asynchronous (○). Animals were fed in two equal meals at 0900 and 1700 h.

Hourly plasma urea concentrations displayed a cyclical trend with peak concentrations occurring approximately 3 h after the morning and afternoon feed (Fig 4.5a). Animals receiving diet FA had significantly higher post-prandial peaks and consistently higher concentrations of plasma urea throughout the day. Synchronising the hourly release of energy and nitrogen (diets SS and FS v. SA and FA) significantly reduced the plasma concentrations of urea 2 hours after the morning feed and tended to reduce plasma levels after the evening meal. A fast rate of energy release in the rumen (diets FA and FS v. SA and SS) tended to raise plasma urea concentrations throughout the day. The interaction between energy release and the degree of synchrony was significantly different for most of the day with animals offered diets SS and FA having higher concentrations of plasma urea than animals fed diets SA and FS respectively.

The diurnal variation in plasma  $\beta$ -hydroxy butyrate is presented in Fig 4.5b. Animals fed diet FA had significantly higher post-prandial  $\beta$ -hydroxy butyrate peak of approximately 1.7 mmol/l compared to values of approximately 0.5 - 0.8 mmol/l in animals fed the other diets (Fig 4.5b). This higher level of plasma  $\beta$ -hydroxy butyrate was sustained throughout the day until just prior to the next meal. Diurnal variation was far less pronounced in animals fed all other rations. A fast release of energy in the rumen (diets FS and FA v. SS and SA) significantly increased plasma concentrations of  $\beta$ -hydroxy butyrate at 1.5, 2.5 and 4.5 h after the morning feed, but significantly decreased it 12.5 and 13.5 h after the evening meal.

Synchronising the hourly release of energy and nitrogen in the rumen significantly reduced peak plasma  $\beta$ -hydroxy butyrate concentrations after the morning feed and levels

tended to be lower after the evening meal. There tended to be less diurnal variation in the plasma  $\beta$ -hydroxy butyrate concentrations in the animals fed synchronous rations.

## 4.7 DISCUSSION

An important criterion of the present study was to supply similar predicted levels of metabolisable energy to the host for all diets. Organic matter digestibilities were similar to those found by other workers (eg Matras *et al.*, 1991) and showed little variation between treatments. AFRC (1992) suggested that the metabolisable energy content of concentrate type feeds can be estimated from the following equation.

$$\text{ME(MJ/kgMD)} = 0.0157(\text{DOMD})$$

where DOMD = digestible organic matter (g/kgDM).

Table 4.5 presents the predicted ME values and ME values calculated from the above equation for the present experiment. Table 4.5 shows that the predicted and calculated ME values are very similar. As the amount of feed offered was based on the ME content the supply of metabolisable energy was also similar for all treatments.

Table 4.5. Predicted and calculated metabolisable energy content (MJ/kgDM) of 4 diets fed to ram lambs.

	Diets <sup>†</sup>				
	SS	SA	FS	FA	SD <sup>‡</sup>
Predicted ME	10.4	10.3	10.6	10.5	0.11
Calculated ME	10.3	10.2	10.6	10.5	0.16

<sup>†</sup>SS = slow energy and synchronous, SA = slow energy and asynchronous, FS = fast energy and synchronous, FA = fast energy and asynchronous.

<sup>‡</sup>Standard deviation

Cocimano and Leng (1967) had observed that high plasma urea concentrations caused by periods of high rumen ammonia levels, increased the amount of nitrogen excreted in urine, therefore reducing nitrogen retention and efficiency. Nitrogen retentions observed in the present study were similar to those found by other workers (Phillips *et al.* 1995; Ahmad *et al.*, 1995; Davenport *et al.*, 1995). However in contrast to Matras *et al.* (1991) no effect of combinations of rates of energy and protein release in the rumen on whole body nitrogen efficiency were found during the present experiment, despite significant differences in diurnal concentrations of plasma urea. The significant difference in total N retention is a reflection of the higher N intakes of animals fed the asynchronous rations. Although the work completed by Matras *et al.* (1991) used isonitrogenous diets the authors made no attempt to measure the supply of metabolisable protein available to the host. Increasing the levels of slowly degradable protein could have increased the supply of digestible undegradable protein, therefore altering nitrogen retentions. Diets formulated for the present study had similar predicted levels of digestible undegradable protein content based on the sum of *in situ* data of the feed ingredients.

An alternative fate for plasma urea, other than excretion from the animal in urine, is to be recycled back to the rumen. Holder *et al.* (1995), using similar diets to Sinclair *et al.* (1993) measured the quantity of nitrogen recycling back to the rumen in animals receiving synchronous or asynchronous supplies of energy and nitrogen to the rumen. Although their results did not reach full significance the authors indicated that a substantial amount of nitrogen was recycled back to the rumen in asynchronous diets. It has also been observed that 40% of urea filtered out in the glomerulus was excreted in ruminants fed protein sufficient diets (Schmidt-Neilsen *et al.*, 1957) and that this level



was reduced to only 1-2% during times of low levels of rumen available nitrogen. As nitrogen retentions were not different between treatments in the present study, but significant differences in plasma urea were observed, it is possible that the nitrogen was recycled to the rumen. The possible effects of this to the metabolism of the animal will be discussed later.

The mean dry matter intake of the lambs on the present study was 0.8 kg per day. Chen *et al.* (1992) studied the effect of dry matter intake on microbial protein production and, at comparable levels of intake, observed very similar microbial nitrogen production per day to the present study. The significantly higher production of microbial nitrogen found when diets promoted a fast release of energy and nitrogen in the present experiment (diet FS) is similar to the increased flows found by McCarthy *et al.* (1989), Herrera-Saldana *et al.* (1990) and Hussein *et al.* (1991). However, apart from the results reported by Herrera-Saldana *et al.* (1990), the increase in microbial protein production observed by these authors did not result in elevated microbial efficiencies.

Plasma concentrations of  $\beta$ -hydroxy butyrate may arise from either  $\beta$ -oxidation of fatty acids of either dietary or tissue origin or from the conversion of butyric acid as it crosses the rumen epithelium (Brockman, 1993). An interesting result from the current experiment is the greater plasma  $\beta$ -hydroxy butyrate concentrations associated with diet FA. Ørskov and Olkjen (1967) observed that carbohydrates with relatively fast rates of fermentation in the rumen increased the proportion of butyrate in the rumen at the expense of acetate. Both diets promoting a fast release of rumen available energy in the present experiment (FS and FA v. SS and SA) significantly increased peaks of plasma

$\beta$ -hydroxy butyrate concentrations after the morning feed. This indicates that differences may exist between treatments in the present study in the production of butyrate in the rumen. However, observations with growing ruminants have noted that during times of protein excess or energy deficiency, body lipid reserves can be mobilised in order to supply ATP required for protein synthesis. This would also give rise to differences in circulating levels of  $\beta$ -hydroxy butyrate due to the  $\beta$ -oxidation of free fatty acids. These observations are an indication that differences in energy metabolism in animals fed the four diets may have existed in the current work.

Ørskov and McCloud (1990) infused varying proportions of propionate to acetate into the rumen of steers, at sufficient levels to support 1.5 times maintenance (energy requirements), whilst maintaining butyrate concentrations. The authors concluded that a deficiency in glucose pre-cursors caused oxidation of protein and gave rise to the elevated plasma  $\beta$ -hydroxy butyrate concentrations observed. Ørskov and McCloud (1990) also suggested that high concentrations of plasma  $\beta$ -hydroxy butyrate in ruminants may therefore be an indication of a shortage of glucogenic precursors as levels were returned to normal by small additions of glucose.

The present experiment did not conclude that a synchronous release of energy and nitrogen in the rumen improved the nitrogen efficiency of the host. There were indications of increased recycling of nitrogen as diets increased in their degree of asynchronicity of energy and nitrogen release in the rumen. Although microbial nitrogen production was increased by diets that combined a fast release of energy and nitrogen to the rumen (diet FS), no overall effect of synchrony on microbial protein production was

established. There are however indications that possible differences existed in energy metabolism between treatments and also between synchronous and asynchronous diets, caused either by rumen fermentation patterns or whole body metabolism.

#### **4.7.1 CONCLUSIONS**

Synchronising the hourly release of energy and nitrogen in the rumen had no effect on nitrogen retention in growing male lambs.

There was an indication that differences in energy metabolism may exist between male lambs offered synchronous or asynchronous diets.

## **4.8 THE EFFECTS OF SYNCHRONISING THE RATE OF ENERGY AND NITROGEN SUPPLY TO THE RUMEN IN DIETS WITH TWO CARBOHYDRATE SOURCES ON GROWTH AND METABOLISM OF MALE LAMBS FED AT A RESTRICTED LEVEL**

### **4.8.1 Introduction**

Although the experiment described in section 4.5 showed that the efficiency of whole body nitrogen capture was not improved by rumen synchrony, possibly due to recycling of urea to the rumen (Holder *et al.* 1995), there were indications of differences in energy metabolism between lambs fed synchronous or asynchronous diets. This potential difference in energy metabolism may cause differences in ruminant productivity. The objectives of the following experiment were to measure the effects of synchronising the hourly rate of energy and nitrogen release in the rumen using diets with two rates of carbohydrate degradation on aspects of rumen and whole body metabolism, growth and carcass characteristics of male lambs fed at a restricted level.

### **4.8.2 Animals and experimental procedure**

Thirty two 10 week old Charollais X Friesland ram lambs weighing *c.* 25 kg were blocked according to live weight and randomly allocated to one of the four dietary treatments described in section 4.2. All lambs were fed on standard lamb creep feed (BOCM, start to finish) from weaning until commencement of the experiment. Animals were adapted to their diets over 7 days by gradual replacement of the start to finish ration

with increasing proportions of the experimental feeds. Lambs were housed individually on raised slatted floor pens and kept under continuous lighting with free access to water. Wool was sheared from the necks of each lamb fortnightly to aid blood sampling. Three weeks after commencing the experiment a lamb, receiving treatment FA, had to be removed from trial due to inappetance. Therefore all results except rumen outflow rates and initial live weight contain one missing value.

#### **4.8.3 Experimental diets**

The diets described in section 4.2 were offered at a restricted level of feeding, sufficient to supply enough metabolisable energy to produce 150 g of live weight gain per day, see section 4.2.1 (AFRC, 1992). Diets were fed in 2 equal portions at 0900 and 1700 h. Animals were weighed weekly at 1100 hours and the amount of food offered was altered to maintain the constant predicted live weight gain of 150 g per day for each individual animal.

#### **4.8.4 Collection and analysis**

##### **4.6.4.1 Feeds**

Samples of all diets were collected fortnightly, dried to a constant weight, pooled and then a sub-sample analysed for organic matter, crude protein, neutral detergent fibre, acid detergent fibre, acid detergent lignin and ether extract (section 2.1). The results are presented in Table 4.3.

#### **4.8.4.2 Live weight**

Lambs were weighed weekly using a stock balance 2 h after the morning feed until they reached the target live weight of 40 kg after which they were slaughtered.

#### **4.8.4.3 Blood collection**

Blood samples (7 ml) were taken into both heparin or potassium oxalate blood tubes (vacutainer) from the jugular vein by venepuncture fortnightly. Samples were taken at 11.00 am.

#### **4.8.4.4 Rumen outflow**

Liquid and solid dilution rates were estimated using cobalt-ethylene diamine tetra acetic acid (EDTA) and chromium mordanted fibre respectively. The markers were administered to 4 blocks of animals (total number of lambs was sixteen).

#### **4.8.4.5 Preparation of the cobalt-EDTA complex**

The cobalt-EDTA complex was prepared as described by Uden *et al.* (1980). 25 g of Co(II) acetate.4H<sub>2</sub>O, 29.2 g cobalt-ethylene diamine tetra acetic acid (EDTA) and 4.3 g LiOH.H<sub>2</sub>O were weighed into a 2 litre beaker containing 200 ml of distilled water. The mix was heated gently until completely dissolved. The solution was then allowed to cool to room temperature and 20 ml of 30% (v/v) hydrogen peroxide added and allowed to

stand for 3 h at room temperature before 300 ml of 95% ethanol (v/v) was added and the solution kept at 5°C overnight. The complex was then filtered using 80% ethanol (v/v) and the filtrate discarded. The crystals were dried at 100°C in an oven overnight and then redissolved in distilled water.

#### ***4.8.4.6 Preparation of the chromium-mordanted fibre***

The Cr-mordanted fibre was prepared as described by Uden *et al.* (1990). To remove soluble plant material a sample of wheat straw was washed at 60°C using a conventional non biological washing powder in a domestic washing machine. The straw was then thoroughly rinsed with tap water and finally acetone (99%; 200 ml) and then dried at 65°C. The fibre was placed in a tin foil tray and a solution of  $\text{Na}_2\text{Cr}_2\text{O}_7$  was poured over it so that the amount of Cr added was between 12-14% of the total fibre weight. The fibre was covered with aluminium foil and baked at 100°C for 24 h. The fibre was then washed thoroughly with tap water before being suspended in tap water and ascorbic acid added (one-half the weight of the fibre). The complex was allowed to stand for 1 h prior to being washed thoroughly with tap water. The material was then dried at 65°C.

#### ***4.8.4.7 Administration of the Co-EDTA and the Cr-mordanted fibre***

Each lamb received a pulse dose (20 ml) of the Co-EDTA complex 1 h before the morning feed at week 10. The Co-EDTA was administered with a drenching gun. Approximately 12 g of the Cr-mordanted fibre was fed to each lamb 1 h before the morning feed. To ensure the lambs would consume the fibre it was mixed with treacle

(5%, w/w) immediately before being offered.

#### **4.8.4.8 Faecal collection**

Faecal grab samples were taken at 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 30, 36, 48, 60, 72, 84, 96 and 108 h after the morning feed. Samples were immediately frozen at -20°C prior to subsequent analysis.

#### **4.8.4.9 Rumen fluid collection**

Samples of rumen fluid were taken using a stomach tube 0, 1, 3, 5 and 7 h after the morning feed as described in section 2.1.12 at week 15. Samples were processed and analysed as described in sections 2.1.12.

#### **4.8.4.10 Carcass component collection**

At slaughter lambs were stunned, bled, skinned and eviscerated. All carcass and non carcass components were collected. Carcasses were weighed within 1 h after slaughter (hot carcass weight) and reweighed after they had been allowed to set at 5°C for 24 h (cold carcass weight). On the day of slaughter the head, feet, genitalia, pelt, liver, lungs plus trachea, total weight of blood and heart were weighed and then discarded. Gut fill was estimated by weighing the intestinal tract before and after the emptying of gut contents.



Twenty four h after slaughter, kidneys and kidney knob and channel fat were removed from the cold carcasses and weighed. Dimensional measurements were taken of the distance from the crown to the rump, gigot circumference and chest depth (Fig 4.6, Brown and Williams, 1979). Carcasses were then halved longitudinally and each half weighed. The right hand side was then cut between the 10<sup>th</sup> and 11<sup>th</sup> rib and the outline of the eye muscle and subcutaneous fat were traced (Fig 4.6). Half carcasses were then frozen at -20°C prior to further processing and analysis.

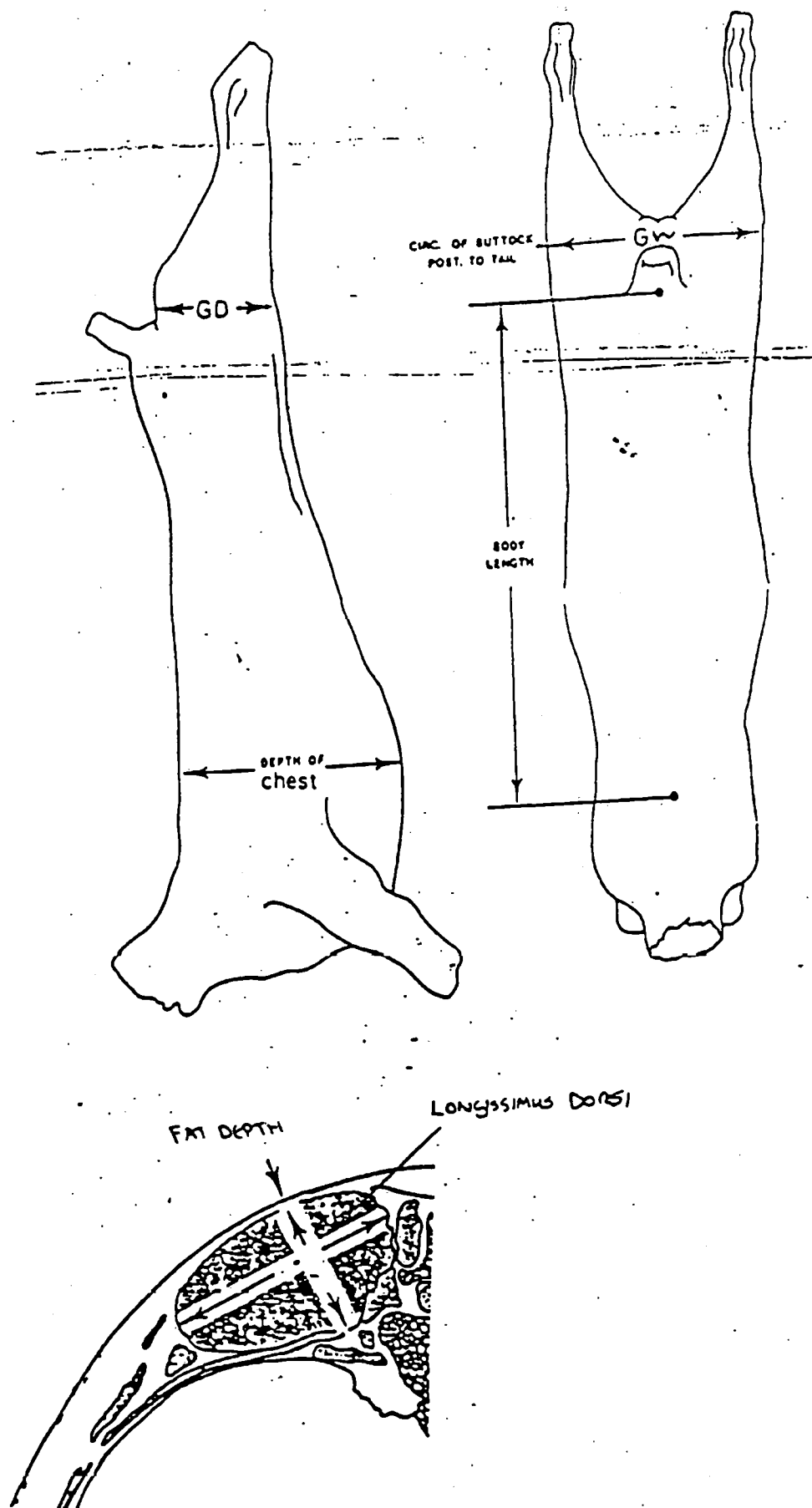


Fig 4.6. Carcass dimensional measurements. (Adapted from Brown and Williams, 1979).

#### **4.8.4.11 Blood analysis**

Blood samples were processed and analysed for urea and BHB content (mmol/l) as described in section 4.5.3.7.

#### **4.8.4.12 Marker analysis**

Approximately 1 g of ground and dried faeces was accurately weighed into a porcelain crucible and then ashed overnight at 550°C in a muffle furnace. The ashed samples were transferred to digestion tubes to which 6 ml of digestion acid (250 ml orthophosphoric acid (85% v/v) added to 250 ml sulphuric acid (98% v/v) and once cool plus 50 ml of manganese solution (10% w/v)) was added. Samples were boiled at 450°C for 20 mins and then allowed to stand at room temperature to cool for approximately 3 mins after which 3 ml of potassium bromate solution (4.5% w/v) was added. Samples were then reheated to 450°C again and reboiled for a further 20 mins before being allowed to cool to room temperature. Once cool the solution was transferred to a 100 ml volumetric flask and diluted to 100 ml with deionised water. The samples were left overnight to allow sediment to settle at the bottom of the flask. Both the chromium and cobalt concentrations of the samples were measured using atomic absorption. Sub-samples were autosampled and concentrations were read at a wavelength of 252.1 nm and 428.9 nm for cobalt and chromium respectively.

Standard solutions of cobalt and chromium were prepared from a working standard solution (SpectrosoL) which contained 1000 ppm of each element. A range of sub-

samples of the working standard (0-10  $\mu$ l and 0-50  $\mu$ l for cobalt and chromium respectively) were diluted to 1 ml with deionised water and then added to 6 ml of the digestion acid (as above) and 3 ml of potassium bromate (as above). The standards were then made up to 100 ml with deionised water and subjected to the same analysis as the faecal samples. Rumen outflow of solid and liquid fractions were estimated as described by Udén *et al.* (1980).

#### **4.8.4.13 Carcass analysis**

Frozen half carcasses were ground through a 13 mm screen and a further 2 times through a 4 mm screen in a whole carcass grinder (Wolfking; Model C160-uni). Samples were thoroughly mixed and sub-sampled (1 kg).

#### **4.8.4.14 Carcass dry matter and organic matter determination**

Sub-samples (50 g fresh material) of ground carcass were taken, frozen and dried in an Edwards Modulyo freeze drier to a constant weight. Samples were analysed for organic matter content as described in section 2.1.2.

#### **4.8.4.15 Crude protein and fat content**

Dried samples were placed in liquid nitrogen until frozen (approximately 15 secs) and then homogenised in a moulinex food processor (model 5302.11). To measure the consistency of this process the organic matter content (g/kgDM) of 10 samples from the

same carcass were determined. The standard deviation suggested that the grinding procedure for carcass samples was satisfactory for the production of consistent ground samples.

To measure the carcass nitrogen content samples were subjected to the automated kjeldahl procedure as described in section 2.1.4. In addition approximately 3 g of homogenized dried sample was accurately weighed into cellulose extraction thimbles which were then plugged with defatted cotton wool. Total fat was then extracted by boiling samples in 40 ml of petroleum ether for 60 mins. Samples were then allowed to rinse for 30 mins. Total fat content was then calculated as described in section 2.1.3.

#### **4.8.5 Statistical analysis**

All results were subjected to a randomised block analysis of variance in a 2 X 2 factorial arrangement. Treatment source was sub-divided into rate of energy release, degree of rumen synchrony between energy and nitrogen release and interaction. Analysis was also performed using initial live weight as a co-variate. All statistical analysis was performed using GENSTAT 5.1 (Lawes Agricultural Trust, 1990).

### **4.9 RESULTS**

There was no significant effect of initial weight as a co-variate in any of the comparisons.

Live weights, live weight gains and feed conversion efficiencies are presented in Table

4.6. There were no significant differences in initial weight or slaughter weight for animals fed any of the diets.

#### 4.9.1 Live weight gain

Growth rates of animals fed the diets described in section 4.2 are presented in Table 4.6. Synchronising the hourly release of energy and nitrogen in the rumen (diets SS and FS v. SA and FA) significantly increased live weight gains of lambs (115.7 g/d v. 97.5 g/d respectively,  $p < 0.05$ ) over the period of growth from 25 kg to 34 kg live weight, the period when metabolisable protein supply was predicted to be deficient. There was no significant difference in growth rate attributable to the main effect of energy or interaction between energy release and synchrony. There was no significant difference in growth rate between treatments from a live weight of 34 kg until slaughter.

Over the whole growth period none of the experimental diets promoted the predicted growth rate of 150 g/d although animals fed synchronous diets approached this (125.7 g/d and 137.4 g/d for diets SS and FS respectively, Table 4.6). From starting live weights until slaughter synchronising the rate of energy and nitrogen release in the rumen (diets SS and FS v. SA and FA) significantly ( $p < 0.001$ ) increased the live weight gain of animals from their initial weight (c. 25 kg) to slaughter weight (131.5 g/d v. 107.0 g/d respectively, Table 4.6). Growth rates, from initial weight until slaughter, were not significantly altered by the rate of release of energy or by the interaction of rate of energy supply and degree of rumen synchronicity (Table 4.6).

#### **4.9.2 Feed conversion efficiency**

During the period of growth from initial live weight (c. 25 kg) until 34 kg synchronising the release of hourly energy and nitrogen (diets SS and FS v. diets SA and FA) significantly increased FCE (0.1347 kg/kg v. 0.1114 kg/kg respectively,  $p < 0.05$ ) over this period. However FCE were not significantly altered by the rate of energy release or by the interaction of energy release and synchrony over this period.

Feed conversion efficiencies from 34 to 40 kg live weight were not significantly affected by the hourly pattern of energy release or the interaction of rate of energy degradation and degree of synchrony in the rumen. Synchronising the hourly release of energy and nitrogen in the rumen (diets SS and FS v. SA and FA) significantly increased the feed conversion efficiency of animals from initial live weight to slaughter weight (0.1385 kg/kg v. 0.1130 kg/kg respectively,  $p < 0.001$ ).

Table 4.6. Mean dry matter intakes, live weights, live weight gains and feed conversion efficiencies of male lambs fed diets in two equal sized meals at 0900 and 1700 h, differing in their rates of energy and nitrogen release in the rumen.

	Diets <sup>a</sup>			Significance of main effects				
	SS	SA	FS	FA	s.e.d.	Energy	Synchrony	Interaction
Dry matter intake (kg)	0.934	0.942	0.915	0.923	0.0564	NS	NS	NS
Initial weight (kg)	25.25	25.25	25.12	25.25	0.302	NS	NS	NS
Slaughter weight (kg)	40.4	40.2	40.8	40.4	0.76	NS	NS	NS
Live weight gain (g/d)								
<i>initial live weight to 34 kg</i>	112.5	95.2	119.0	99.9	9.91	NS	*	NS
<i>34 kg to slaughter</i>	147.5	134.3	161.4	123.6	21.01	NS	NS	NS
<i>initial to slaughter</i>	125.7	108.3	137.4	106.5	8.09	NS	***	NS
FCE (kg gain/kg DMI)								
<i>initial live weight to 34 kg</i>	0.1293	0.1067	0.1400	0.1161	0.01311	NS	*	NS
<i>34 kg to slaughter</i>	0.1405	0.1283	0.1582	0.1200	0.02302	NS	NS	NS
<i>initial to slaughter</i>	0.1324	0.1140	0.1446	0.1121	0.00852	NS	***	NS

<sup>a</sup>SS = Slow release of energy and synchronous, SA = slow release of energy and asynchronous, FS = fast release of energy and synchronous, FA = fast release of energy and asynchronous.

NS = not significant, \* = p<0.05, \*\*\* = p<0.001  
error degrees of freedom = 20 (1 missing value)



### **4.9.3 Blood metabolites**

So that comparisons of plasma metabolite concentrations could be made for animals at the same physiological stages of growth, plasma concentrations of urea and BHB were compared between animals at the same live weights.

#### **4.9.3.1 Blood urea concentration**

Plasma urea concentrations for animals fed diets varying in their predicted rate of energy and nitrogen release in the rumen are presented in Fig. 4.7a. Plasma urea concentrations were consistently higher in lambs fed diet SS than all other treatments, this effect being significant ( $p < 0.05$ ) at 37 kg live weight (Fig 4.7a). In addition plasma urea concentrations increased in animals fed the SS diet as live weight increased but this trend was not apparent in any animals fed any other diet (Fig 4.7a).

There was a significant interaction between rate of energy release and synchrony with animals fed diets SS and FA having higher plasma urea levels at a live weight of 31, 32 and 40 kg. Synchronising the hourly release of energy and nitrogen in the rumen (diets SS and FS v. diets SA and FA) tended to increase the concentration of plasma urea in lambs (Fig 4.7a). This higher concentration was significant at a live weight of 37 kg (7.508 mmol/l v. 6.625 mmol/l respectively,  $p < 0.01$ ).

#### 4.9.3.2 Blood $\beta$ -hydroxy butyrate concentration

Plasma BHB concentrations during growth for animals fed diets varying in their predicted rate of energy and nitrogen release in the rumen are presented in Fig. 4.7b. From a live weight of 31 kg until slaughter weight (40 kg) animals fed diet FA had significantly elevated concentrations of plasma BHB ( $p < 0.05$ ). Concentrations of plasma BHB in animals fed the other diets did not significantly differ and remained relatively constant throughout the growth period (Fig 4.7b). Synchronising the release of energy and nitrogen in the rumen (SS and FS v. SA and FA) significantly lowered the concentration of plasma BHB after animals reached 31 kg live weight ( $p < 0.05$ ).

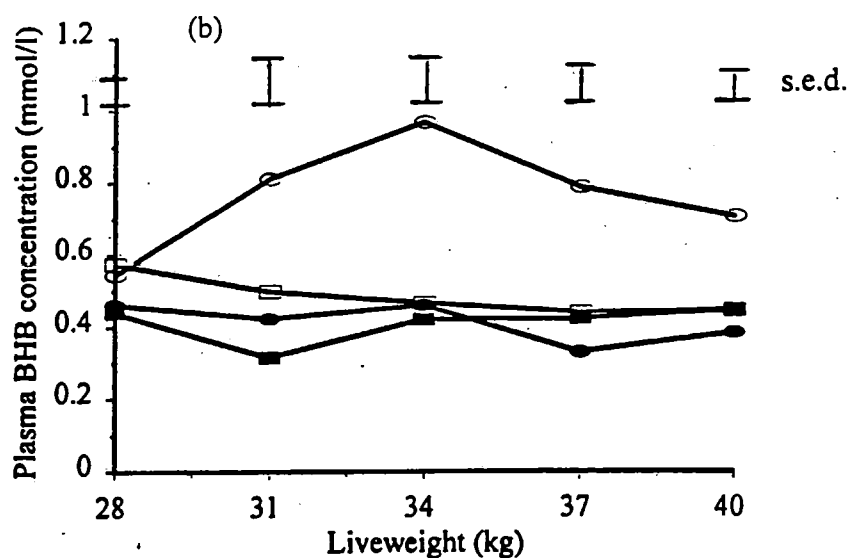
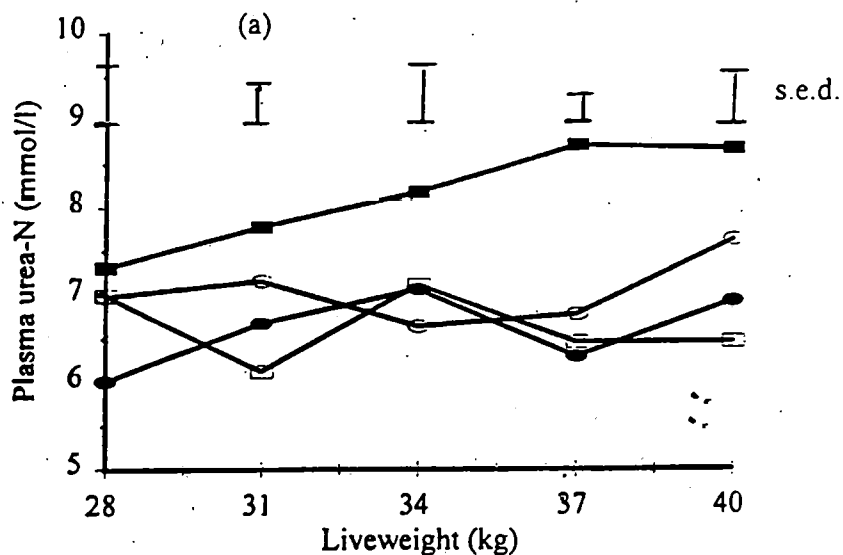


Fig 4.7. Mean plasma concentrations of (a) urea and (b)  $\beta$ -hydroxy butyrate 2 h after the morning feed from 28 kg to 40 kg live weight of male lambs fed diets formulated to have either a slow release of energy and synchronous (■), slow energy and asynchronous (□), fast energy and synchronous (●) or fast energy and asynchronous (○). Animals were fed in two equal meals at 0900 and 1700 h.

#### 4.9.4 Carcass components

Carcass measurements for lambs fed synchronous or asynchronous diets are presented in Table 4.7. Hot carcass weight, cold carcass weight, killing out proportion, gigot circumference, gigot depth, body length, chest depth, depth of subcutaneous fat, *longissimus dorsi* area, carcass dry matter, carcass ash, carcass fat and carcass nitrogen were not significantly affected by treatment (Table 4.7).

Hourly release of energy in the rumen did not significantly alter the amount of kidney, knob and channel fat (Table 4.7) but a synchronous release of energy and nitrogen in the rumen (SS and FS v. SA and FA) significantly increased these deposits (264 g v. 205 g respectively,  $p < 0.01$ ). In addition, although not fully significant animals fed synchronous diets tended to have a thicker layer of subcutaneous fat surrounding the *longissimus dorsi* muscle (3.09 mm v. 2.56 mm respectively, Table 4.7).

#### 4.9.5 Non carcass components

The non carcass measurements made on animals fed diets differing in their predicted rate of energy and nitrogen release in the rumen are presented in Table 4.8. There was no significant difference in rumen contents, intestine weight or intestine contents ( $p > 0.05$ ). Synchronising the rate of hourly release of energy and nitrogen in the rumen (diets SS and FS v. SA and FA) significantly increased the weight of the rumen (1.708 kg v. 1.554 kg respectively,  $p < 0.05$ ).

Table 4.7. Carcass components of male lambs fed diets in two equal meals at 0900 and 1700 h, differing in the hourly release of energy and nitrogen in the rumen.

	Diets*	Significance of main effects						
		SS	SA	FS	FA	s.e.d.	Energy	Synchrony
Hot carcass weight (kg)	19.28	19.57	19.91	18.73	0.698	NS	NS	NS
Cold carcass weight (kg)	18.36	18.66	18.70	18.12	0.511	NS	NS	NS
Killing out proportion	0.4410	0.4375	0.4415	0.4375	0.12850	NS	NS	NS
Gigot circumference (cm)	63.28	63.26	62.03	61.40	1.198	NS	NS	NS
Gigot depth (cm)	14.31	14.01	14.13	14.45	1.048	NS	NS	NS
Body length (cm)	49.27	49.50	51.08	49.15	1.325	NS	NS	NS
Chest depth (cm)	14.31	14.01	14.13	14.45	1.048	NS	NS	NS
Kidney fat (g)	278	203	251	208	29	NS	**	NS
Depth of subcutaneous fat (mm)	2.81	2.36	3.37	2.75	0.746	NS	NS	NS
<i>Longissimus Dorsi</i> area (cm <sup>2</sup> )	12.03	11.74	11.46	11.78	0.930	NS	NS	NS
Carcass dry matter (g/kg)	386	388	399	393	8.9	NS	NS	NS
Carcass ash (g/kgDM)	129	139	133	123	9.6	NS	NS	NS
Carcass fat (g/kg DM)	404	396	417	424	19.8	NS	NS	NS
Carcass nitrogen (g/kgDM)	72.71	71.70	70.03	68.86	2.450	NS	NS	NS

<sup>a</sup>SS = Slow release of energy and synchronous, SA = slow release of energy and asynchronous, FS = fast release of energy and synchronous, FA = fast release of energy and asynchronous.

NS = not significant, \* =  $p < 0.05$ , means with different superscripts are significantly different ( $p < 0.05$ ).

Error degrees of freedom = 20 (1 missing value)

Table 4.8. Non carcass components of male lambs fed diets in two equal meals at 0900 and 1700 h, differing in the hourly release of energy and nitrogen in the rumen.

	Diets <sup>*</sup>				Significance of main effects			
	SS	SA	FS	FA	s.e.d.	Energy	Synchrony	Interaction
Rumen (kg)	1.729	1.555	1.686	1.554	0.0962	NS	*	NS
Intestine (kg)	1.650	1.690	1.620	1.507	0.0948	NS	NS	NS
Rumen contents (kg)	5.00	5.30	5.12	5.71	0.364	NS	NS	NS
Intestinal contents (kg)	1.394	1.424	1.279	1.386	0.1365	NS	NS	NS

<sup>\*</sup>SS = slow hourly energy and nitrogen release, SA = slow hourly energy and fast nitrogen release, FS = fast hourly energy and fast nitrogen release, FA = fast hourly energy and slow nitrogen release.

NS = not significant, \* =  $p < 0.05$ .

Error degrees of freedom = 20 (1 missing value)

#### **4.9.6 Rumen metabolism**

The effects of varying the hourly rate of release of energy and nitrogen in the rumen on rumen metabolism are presented in Table 4.9.

##### **4.9.6.1 Rumen fluid pH**

The mean rumen fluid pH values for animals fed diets differing in their predicted release of energy and nitrogen in the rumen are presented in Table 4.9. A fast release of energy in the rumen significantly reduced the mean rumen fluid pH (Table 4.9,  $p < 0.01$ ). Similarly, synchronising the release of energy and nitrogen in the rumen significantly reduced mean rumen fluid pH (Table 4.9,  $p < 0.01$ ). There was no significant interaction between rate of energy release and rumen synchrony on mean rumen fluid pH.

##### **4.9.6.2 Rumen outflow rates**

Mean solid and liquid outflow rates for lambs fed synchronous or asynchronous diets are presented in Table 4.9. There was no significant difference between treatments for either solid or liquid rates of passage from the rumen (Table 4.9) with mean values of 0.025 and 0.055 for the solid and liquid passage rates respectively.

#### **4.9.6.3 Total volatile fatty acid concentration and proportions of individual volatile fatty acids**

Mean total volatile fatty acid concentrations are presented in Table 4.9. A slow release of energy significantly increased the TVFA concentrations ( $P < 0.05$ ) as did synchronising the hourly release of energy and nitrogen in the rumen ( $p < 0.001$ , Table 4.9). There was no significant interaction between rate of energy release in the rumen and synchrony on mean TVFA concentrations (Table 4.9).

A slow release of energy in the rumen significantly raised the molar proportions of acetic acid in the rumen (Table 4.9,  $p < 0.001$ ) and significantly lowered ( $p < 0.01$ ) the molar proportions of propionic acid. There was a significant interaction ( $p < 0.05$ ) between rate of energy released in the rumen and synchrony on mean molar proportions of valeric acid with animals fed diet FS having lower proportions than animals fed diet FA. No other effects of treatment was seen on mean VFA proportions.



Table 4.9. Mean rumen fluid pH, rumen outflow rates and rumen fluid concentrations of total volatile fatty acids, and molar proportions of acetic acid, propionic acid, butyric acid, iso-butyric acid, iso-valeric acid, valeric acid and caproic acid in male lambs fed diets in two equal meals at 0900 and 1700 h, differing in the hourly release of energy and nitrogen in the rumen.

	Diets <sup>a</sup>		Significance of main effects					
	SS	SA	FS	FA	s.e.d.	Energy	Synchrony	Interaction
Rumen fluid pH <sup>1</sup>	6.26	6.46	6.14	6.33	0.057	**	**	NS
Rumen outflow (per h)								
<i>solid</i>	0.022	0.027	0.025	0.025	0.0042	NS	NS	NS
<i>liquid</i>	0.052	0.060	0.058	0.049	0.0057	NS	NS	NS
Total volatile fatty acid (mmol/l) <sup>1</sup>	48.0	24.0	32.0	24.0	5.25	*	***	NS
Acetic acid (mmol/mol) <sup>1</sup>	738	730	691	711	11.37	***	NS	NS
Propionic acid (mmol/mol) <sup>1</sup>	190	200	252	225	16.94	**	NS	NS
Butyric acid (mmol/mol) <sup>1</sup>	66	65	52	66	4.9	NS	NS	NS
Iso-butyric acid (mmol/mol) <sup>1</sup>	0.81	0.06	0.59	0.68	0.327	NS	NS	NS
Valeric acid (mmol/mol) <sup>1</sup>	1.98	1.79	1.65	2.42	0.308	NS	NS	*
Iso-valeric acid (mmol/mol) <sup>1</sup>	1.57	0.58	1.27	1.17	0.546	NS	NS	NS
Caproic acid (mmol/mol) <sup>1</sup>	1.84	1.64	1.30	1.88	0.415	NS	NS	NS

<sup>a</sup>SS = slow hourly energy and nitrogen release, SA = slow hourly energy and fast nitrogen release, FS = fast hourly energy and nitrogen release, FA = fast hourly energy and slow nitrogen release.

NS = not significant, \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ .

Error degrees of freedom = 20 (1 missing value)

## **4.10 DISCUSSION**

### **4.10.1 Rumen retention**

The effective degradabilities of both organic matter and nitrogen are dependant on the rate of exit of material from the rumen. Outflow rates chosen in order to formulate rations for the current experiments were based on the level of feeding (AFRC, 1992) and because this was similar for each treatment predicted outflow rates were similar also. Although measured outflow rates were not significantly different between treatments they varied slightly and were lower than the predicted value. The lower outflow rates obtained suggest that the actual level of feeding (in relation to maintenance energy requirements) was lower than that predicted (AFRC, 1990) and this is substantiated by the growth rates observed as they were below the expected rate of 150 g/d.

There are a number of factors affecting the passage of nutrients from the rumen. Poncet (1991) during his review of factors affecting rumen outflow rate included rumen motility (itself a function of feeding behaviour and external temperature), level of feeding, particle size and density, distribution of particles within the rumen, selective retention of particles, influx and efflux of water, diet type, feed processing, physiological status of the animal, age of the animal, defaunation, species, mixing rate of the reticulorumen, breakdown rate of particles, entrapment of particles in the fibre mat, mineral salts, photoperiod and animal size. The equation quoted by AFRC (1992) for the estimation of rumen outflow rate is based only on level of feeding and could conceivably be affected by any or all of the above factors quoted above resulting in different outflows than those

expected.

As observed rumen outflows were different than those predicted a question arises as to what effect this would have on the pattern of degradation of energy and nitrogen yielding substrates contained in the rations. The SIRE formulation program utilizes the solid outflow rate (Sinclair, 1992) and Table 4.10 compares rumen degradability parameters of the four diets using an outflow of 0.046/h (the outflow used to originally formulate the ration) or the mean solid outflow as measured for each treatment.

Table 4.10. Comparison of rations formulated with either the predicted outflow rate of 0.046 or the measured rumen outflows. Figures are based on animals fed 1000 g DM per 24 h split into two equal meals 8 h apart.

Outflow/h	Diets <sup>†</sup>							
	SS		SA		FS		FA	
	0.046	0.022	0.046	0.027	0.046	0.025	0.046	0.025
Organic matter degraded (g/kgDM)	439	527	426	498	536	583	520	567
Nitrogen degraded (g/kgDM)	14.0	16.0	16.0	17.1	16.0	17.5	18.0	19.0
Organic matter degradability	0.49	0.59	0.47	0.55	0.58	0.63	0.58	0.63
Nitrogen degradability	0.63	0.72	0.67	0.72	0.74	0.79	0.77	0.80
Daily ratio OM:N (gN/kgOMTDR)	32	31	37	34	31	30	35	33
Metabolisable Protein (g/kgDM)	87	81	88	86	84	79	84	76
Digestible undegradable protein (g/kgDM)	37	26	35	28	28	22	26	22
Synchrony index	0.84	0.84	0.54	0.56	0.87	0.88	0.51	0.53

<sup>†</sup>SS = slow energy and synchronous, SA = slow energy and asynchronous, FS = fast energy and synchronous, FA = fast energy and asynchronous.

Decreasing the rate of passage from the rumen is predicted to increase particle retention time and therefore increase the effective degradability (Ørskov and McDonald, 1979). This is clearly shown in Table 4.10 as organic matter and nitrogen degraded (g/kgDM) and organic matter and nitrogen degradabilities (g/g) increased as rumen outflow was decreased. Conversely levels of digestible undegradable protein were reduced. Due to these decreased levels of digestible undegradable protein a greater reliance is placed upon microbial protein to supply digestible amino acid nitrogen. Table 4.10 indicates that the daily ratio of nitrogen to organic matter degraded (gN/kgOMTDR), although slightly lower than predicted remains similar between treatments. Similarly, the synchrony indices are relatively unaffected by the change in outflow and therefore provides confidence that the diets behaved as predicted.

#### 4.10.2 Growth

The lack of response of growth and feed conversion efficiencies to the rate of degradation of energy or the interaction between energy release and degree of rumen synchrony in the present study is similar to results observed by others (Hussein and Jordan, 1991; Beauchamin *et al.*, 1995). Hussein and Jordan (1991) supplemented a basal ration of alfalfa and corn with varying levels of soya bean meal and fishmeal, ranging from 0-100 % replacement. The authors found that the rate of release of rumen degradable nitrogen had no significant effect on lamb growth rates or feed conversion efficiencies. In agreement Matras *et al.* (1991) found no effect of rate of energy degradation on lamb performance.

Similarly to Matras *et al.* (1991), the results of the current experiment showed that lamb growth rates and feed efficiencies benefit from diets formulated from ingredients that have similar patterns of nitrogen and organic matter degradability. It is tempting to suggest that the improvement in animal performance was due to improvements in efficiency of nitrogen metabolism in the rumen as has been observed by others (e.g. Sinclair *et al.*, 1993; Sinclair *et al.*, 1995; Matras *et al.*, 1991; Aldrich *et al.*, 1993; Herrera-Saldana *et al.*, 1990; Rooke *et al.*, 1987; Khalili and Huhtanen, 1991a; Newbold and Rust, 1992) when comparing synchronous to asynchronous type rations. However observations made during the metabolism trial of the current work where the same diets, fed at the same level of feeding to the same weight and breed of lambs did not result in increased nitrogen retention. Therefore an alternative explanation is required for the significantly increased feed conversion efficiencies observed.

Barcroft *et al.* (1944) showed that the main source of energy to ruminants was volatile fatty acids. Ørskov and MacLeod (1990) suggested that the differences in efficiency of utilisation between glucogenic and ketogenic volatile fatty acids was simply due to increased heat production associated with fibre degradation. Ørskov and Macleod (1990) suggested this results in less energy available to the host when acetate promoting feeds (fibrous) make up a substantial part of the ration. In the present study acetate and propionate proportions were increased and decreased respectively by higher and lower contents of neutral detergent fibre. However, in the current work, rate of energy release had no significant effect on growth.

Ørskov and MacLeod (1990) suggested that elevated plasma  $\beta$ -hydroxy butyrate

concentrations, as observed in the metabolism trial with diet FA, may be due to a lack of glucose precursors. The supply of glucose to the ruminant animal includes intestinal digestion of rumen bypass starch or hepatic metabolism of propionic acid and other glucose precursors such as certain amino acids. Carbohydrate sources for the present experiment were either barley, tapioca, winter wheat straw or unmolassed sugar beet pulp. The rates of degradation for barley and tapioca used in the present experiment (Table 3.3) at the outflow rates observed give organic matter effective degradabilities of 83% and 84% respectively and are therefore unlikely to supply appreciable amounts of bypass starch. Similarly the starch content of winter wheat straw and sugar beet pulp was zero (Table 3.1) and therefore intestinal supply of glucose from these ingredients would be zero. The only sources of glucose precursors available therefore are from propionate produced in the rumen and large intestine and glucogenic amino acids.

Although microbial nitrogen production was significantly higher in lambs fed diet FS during the metabolism trial an effect of synchrony on microbial protein production was not observed. As outflow rates did not vary between treatments it is probable that there was no effect of synchrony on metabolisable protein (microbial protein plus digestible undegradable protein) supply although the enhancement in animal performance of animals fed diet FS may have been due to a greater digestible protein supply.

MacRae (1985) suggested that the efficient utilization of ketogenic precursors depends on their conversion to fatty acids and then into triacylglycerides. These metabolic steps require the reducing equivalent  $\text{NADPH}^+$  produced either via glycolysis or from isocitrate dehydrogenase (Krebs cycle) when glucose supplies are limiting (Stryer, 1988).

Production of NADPH<sup>+</sup> via the iso-citrate pathway will have two effects on energy metabolism. First, the Krebs cycle must be fuelled which ultimately means less acetate available for tissue growth and secondly, NADPH<sup>+</sup> production from iso-citrate requires an uncoupling of metabolism which increases heat loss. The present study observed not only slight increases in glucogenic volatile fatty acid proportions due to synchrony but also significantly elevated total volatile fatty acid levels, indicating that animals fed asynchronous diets may have been limited in glucose pre-cursors.

There are other areas of possible increase in ATP usage leaving less energy for host metabolism. The transfer of plasma urea to the rumen was described by Cheng and Wallace (1979) as an active process requiring an input of ATP. In addition, the secretion of bacterial ureases will be costly in terms of ATP catabolized. McBride and Kelly (1990) found that detoxification of plasma ammonia by the liver, originating from the rumen, is a major use of ATP. These requirements for ATP will probably be higher in diets that promote an increased plasma urea concentration as observed with the animals fed the fast energy asynchronous diet in the present study. Under substrate limited environments bacterial heat production has been seen to be excessive but with no increase in microbial growth (Russell and Wallace, 1988) resulting in futile cycles. The asynchronous diets in the present study were predicted to be limiting in protein supply to the rumen at various hours of the day which could have increased heat production and altered bacterial composition (Nocek and Russell, 1988) resulting in less energy available to the host.

Lobley (1992) and MacRae (1985) have suggested that under energy limiting conditions, protein catabolism may occur to supply essential glucogenic precursors. The possible

energy costs of nitrogen recycling, detoxification of plasma ammonia by the liver, futile cycles by rumen bacteria, increased heat production due to acetate clearance and production of NADPH<sup>+</sup> and less production of volatile fatty acids promoted by asynchrony could conceivably lower energy available to the host receiving asynchronous diets. In an attempt to capitalise on the resultant decreased supply of energy, a proportion of metabolisable protein could have been oxidised leaving less energy and protein available for growth and consequently resulting in the lower growth rates observed. However Ørskov and MacCloud (1990) suggested that increased protein oxidation would result in an accumulation of amino groups which is likely to increase the excretion of nitrogen in urine. However, increased urinary N was not recorded in the metabolism experiment although animals receiving asynchronous diets may have already been recycling nitrogen back to the rumen (as observed by Holder *et al.* (1995)), and so conceivably instead of being excreted this extra nitrogen was recycled and possibly recaptured as microbial protein at a later time.

All ram lambs had slower growth rates and lower feed conversion efficiencies below 34 kg than above this weight. O'Donovan (1984) observed compensatory growth in animals offered ample food supplies, previously being undersupplied with nutrients. In the present study the predicted under supply of protein for all animals below 34 kg live weight may have caused a similar effect to those observed by O'Donovan (1984), and once the supply of energy and protein was sufficient (above 34 kg) compensatory growth may have occurred. This was particularly apparent in animals fed diet SA and therefore may have reduced the impact of synchrony on growth and feed conversion efficiency from 34 kg onwards.



#### 4.10.3 Carcass and non carcass characteristics

Lambs of the same genotype and sex provided with sufficient energy and protein slaughtered at the same weight will have a similar body composition (Buttery *et al.* 1990). The types of “balanced” rations spoken of by Cropper (1989) supply a certain ratio of metabolisable protein to metabolisable energy to give sufficient metabolites to sustain a certain live weight gain. Table 4.11 suggests 8 dietary scenarios for various metabolisable protein to metabolisable energy ratios and observed effects on carcass composition and growth. Implications thus far are that the combination of varying rates of energy and protein to result in asynchronous diets were limiting in energy supply. Theoretically the asynchronous diets of the present study may have resulted in high MetP:MetE fed at a restricted level of feeding as described in scenario 6 (Table 4.11). The corresponding prediction is that under these conditions lambs would grow at their protein potential but not fatten. However, in the present study, no differences were observed between treatments for carcass contents of fat or protein. However if a proportion of metabolisable protein was being oxidised due to urea synthesis, as suggested by Lobley (1992), energy and protein ratios could have been similar between synchronous and asynchronous rations but absolute amounts lower for animals fed asynchronous diets. This would support the lower growth rates observed and the lack of differences found in carcass fat and protein content.

Table 4.11. The predicted growth responses of 25 kg lambs capable of 43 g of protein and 94 g of lipid deposition per day offered various diets supporting different metabolisable protein (MetP) and Metabolisable energy (MetE) supplies to the animal.

	Diet	Prediction	Evidence and comments
1	Balanced MetP to MetE ratio offered <i>ad lib</i> .	Lambs grow at their potential rate.	Feeding a "balanced" diet is difficult as required ratio of MetP to MetE becomes progressively less as animals grow. Choice feeding studies by Cropper <i>et al.</i> (1986) suggest lambs capable of selecting diets to provide 10g DCP/MJME.
2	High MetP to MetE ratio offered <i>ad lib</i> .	Grow protein at potential and fatten normally.	Emmans <i>et al.</i> (1987). Assumes diets of sufficient energy density to enable lambs to consume sufficient MetE to meet requirements. Lambs required to eat greater amount than in option 1 due to ATP needed for deamination of surplus protein.
3	Low MetP to MetE ratio offered <i>ad lib</i> .	Grow protein at potential but fatten excessively.	Ranhotra and Jordan (1966). Lambs eat to meet their MetP requirements but in so doing consume MetE above requirements. Assumes diets of sufficient energy density to enable lambs to consume sufficient MetE to meet requirements.
4	Low MetP to MetE ratio offered <i>ad lib</i> in a hot environment.	Limited in protein growth.	Ames and Brink (1977). Intake is lowered due to warm environmental conditions. Possibly lambs excessively fatten as less energy can be dissipated as heat. This situation suggests that requirements for balanced rations changes as external environment changes.
5	Low MetP to MetE ratio offered <i>ad lib</i> in a cold environment.	Grow at protein potential and fatten normally.	Ames and Brink (1977). Additional MetE is required due to low external temperatures. This situation suggests that requirements for balanced rations changes as the external environment changes.
6	High MetP to metE ratio offered at restricted intake.	Grow at protein potential but do not fatten.	Energy is limiting to growth. Under such conditions amino acids utilised for energy (Black <i>et al.</i> , 1990; Kelly <i>et al.</i> 1993). Lobley (1992) suggested that under certain energy limited situations 50 - 100% of energy supply could originate from glucogenic precursors.
7	Medium MetP to MetE ratio offered at restricted intake.	Both fattening and protein growth limited.	Fattet <i>et al.</i> (1984). Animals not able to consume sufficient amounts of feed to enable them to reach their potential growth rates.
8	Low MetP to MetE ratio offered at restricted intake.	Limited protein growth and fatten excessively.	Andrews and Ørskov (1970). MetP intake is above requirements to support normal body to lipid protein ratio. Excess energy is stored as essential body lipid.

Adapted from Cropper (1989).

Johnson *et al.* (1990) concluded that the weight of the gastro-intestinal tract and liver increased or decreased with changing level of feeding on an energy basis. In addition fat is expendable during periods of restricted nutrient intake. Although neither the rate of energy or protein supply alone altered intestine weight or fat deposition, combinations to provide rations that were synchronous for the hourly release of energy and nitrogen in the rumen, significantly increased the deposition of fat around the kidneys and also significantly increased the weight of the rumen, further indications of differences in energy metabolism between animals fed synchronous and asynchronous diets.

#### **4.10.4 Blood metabolites**

Blood sampling for the current experiment took place 2 hours after the morning feed. The results indicate that, at this time plasma urea levels were not affected by synchrony throughout the growth period. Peak plasma urea concentrations were seen ranging from 3-5 h after feed had been offered during the metabolism trial (see section 4.4) and it is therefore possible that peak concentrations in the present experiment were missed.

Circulating concentrations of  $\beta$ -hydroxy butyrate in the present study are in direct confirmation with the patterns observed during the metabolism trial (section 4.4). Although some variation existed in the proportions of butyrate in the rumen between individual diets at various times after feeding no differences were observed due to synchrony. The explanation that differences in plasma  $\beta$ -hydroxy butyrate observed in animals fed asynchronous diets during the metabolism trial (section 4.4) was due to differences in rumen butyrate concentrations seems unfounded. Because body lipid

concentrations were not different between treatments at slaughter suggests that the elevated plasma  $\beta$ -hydroxy butyrate levels were not caused by a mobilisation of body reserves as suggested by Brockman (1993). The elevated  $\beta$ -hydroxy butyrate may therefore be evidence in support of a glucose precursor deficiency as suggested by Ørskov and MacCloud (1990).

#### **4.10.5 CONCLUSIONS**

Synchronous rations fed at a restricted level of feeding increased the growth rate and feed conversion efficiency of male lambs compared to those fed asynchronous diets.

The observed increase in growth rate of lambs fed synchronous rations may be due to differences in energy metabolism.

## **4.11 THE EFFECTS OF SYNCHRONISING THE RATE OF ENERGY AND NITROGEN SUPPLY TO THE RUMEN IN DIETS WITH TWO CARBOHYDRATE SOURCES ON GROWTH AND METABOLISM OF EWE LAMBS FED *AD LIBITUM***

### **4.11.1 Introduction**

The experiment described in section 4.8 observed that lambs fed synchronous rations at a restricted level of feeding had faster growth rates and feed conversion efficiencies compared to those fed asynchronous diets. Under most commercial feeding situations for growing ruminants, rations are offered *ad libitum* in order to fully exploit the productive potential of the animal. Under these conditions animals have the opportunity to alter their pattern of feed intake which has the potential to alter the actual degree of synchrony of nutrient release in the rumen.

The objective of this experiment was to assess the effects on growth, rumen and body metabolism, feed intake and carcass characteristics of ewe lambs fed the rations described in section 4.2 *ad libitum*.

### **4.11.2 Animals and experimental procedure**

Twenty four 10 week old Charolais X Friesland ewe lambs weighing c. 25 kg were blocked according to live weight and randomly allocated to one of the four dietary treatments (section 4.2). Animals were housed individually on raised slatted floor pens

and kept under continuous lighting with free access to water. Wool was sheared from the necks of each lamb fortnightly to aid blood sampling.

#### **4.11.3 Experimental diets**

The diets described in section 4.2 were offered *ad libitum* (115 % of intake). Fresh food was offered daily at 09.00 h and refusals were weighed, sub-sampled and then discarded every second day. The amount of food offered was based on the measured refusal rates.

#### **4.11.4 Collection and analysis**

##### **4.11.4.1 Feeds**

Samples of all diets were collected weekly, dried to a constant weight, pooled and then analysed for organic matter, crude protein, neutral detergent fibre, acid detergent fibre, acid detergent lignin and ether extract (section 2.1). The results are presented in Table 4.3.

##### **4.11.4.2 Live weight**

Lambs were weighed weekly 2 h after their food had been offered using a stock balance. Once lambs had reached the target live weight of 40 kg they were slaughtered.

#### **4.11.4.3 Blood collection**

Blood samples (7 ml) were taken from the jugular vein by venepuncture every 4 h for a 24 h period at week 7 into blood tubes containing either heparin or potassium oxalate. In an attempt to prevent any adverse effects on feed intakes due to the stress of sampling, blood samples were obtained over a 3 day period.

#### **4.11.4.4 Rumen fluid collection**

Samples of rumen fluid were taken using a stomach tube at 0, 1, 3, 5 and 7 h after fresh feed had been offered as described in section 2.1.12. Samples were taken at week 8. In an attempt to prevent reduced feed intakes by stress associated with the sampling process, rumen fluid samples were collected over a 3 day period. Samples were processed and analysed as described in section 2.1.12.

#### **4.11.4.5 Carcass component collection**

Carcass and non carcass components were collected and analysed as described in section 4.8.4.10.

#### **4.11.4.6 Blood analysis**

Blood samples were processed and analysed as described in section 4.8.4.3 and 4.5.4.7.



#### **4.11.4.7 Carcass analysis**

Carcass processing and analysis was carried out as described in section 4.8.4.13.

#### **4.11.4.8 Statistical analysis**

All results were subjected to a randomised block analysis of variance in a 2 X 2 factorial arrangement. Treatment source was sub-divided into energy, nitrogen and interaction. Analysis was also performed using initial live weight as a co-variate. All statistical analysis was performed using GENSTAT 5.1 (Lawes Agricultural Trust, 1990).

### **4.12 RESULTS**

Initial weight was used as a co-variate but was not significant for any of the parameters monitored.

Mean live weights, live weight gains, feed intakes and feed conversion ratios are presented in Table 4.12. There was no significant difference in the initial live weight of animals fed any of the diets. However, animals fed the synchronous diets were approximately 0.6 kg heavier than animals offered the asynchronous rations at slaughter ( $p < 0.05$ , Table 4.12).

#### **4.12.1 Live weight gain**

For the period of growth from initial weight (c. 25 kg) until 34 kg live weight, the time after which metabolisable protein was predicted to be in excess there was no significant main effect of rate of release of energy (diets SS and SA v. FS and FA) or synchronisation of the rate of energy and nitrogen release in the rumen (SS and FS v. SA and FA). However, there was a significant interaction between energy release and synchrony in that animals offered diet SS grew significantly faster than animals offered diet SA from initial live weight to 34 kg ( $p < 0.05$ ). From a live weight of 34 kg until slaughter there was no significant interaction between rate of release of energy and the degree of synchrony.

A fast release of energy in the rumen (diets FA and FS) significantly increased growth rates from initial live weight to slaughter compared to diets supplying a slow rate of release of energy in the rumen (diets SS and SA; 265.7 g/d v. 230.0 g/d respectively,  $p < 0.05$ ; Table 4.12). Neither the degree of hourly synchronisation of energy and nitrogen in the rumen or the interaction of rate of energy release and synchrony significantly altered animal growth rates over this period (Table 4.12).

#### **4.12.2 Feed intake**

There was no significant effect on feed intake of either the rate of release of energy in the rumen or synchronising the hourly release of energy and nitrogen in the rumen. However there was a significant interaction between energy release and synchrony and animals

offered diet FA ate significantly more feed than animals offered diet FS (Table 4.12).

#### **4.12.3 Feed conversion efficiency**

For the period of growth from initial live weight to 34 kg there was no significant effects of rate of release of energy, synchrony or interaction of energy release and synchrony. From a live weight of 34 kg to slaughter, there was no significant main effects, however there was a significant interaction and animals offered diet FS had significantly ( $p < 0.05$ ) higher FCE than animals offered diet FA (Table 4.12). Over the total growth period (initial to slaughter) a fast release of energy in the rumen significantly increased ( $p < 0.001$ ) the FCE as did synchronising the release of energy and nitrogen in the rumen ( $p < 0.05$ , Table 4.12). No significant interaction was observed for FCE of the period of growth from initial live weight until slaughter.

Table 4.12. Mean initial live weight, live weight gain, feed intake and feed conversion efficiency of ewe lambs fed diets differing in their rates of energy and nitrogen release in the rumen *ad libitum*.

	Diets <sup>a</sup>		Significance of main effects					
	SS	SA	FS	FA	s.e.d.	Energy	Synchrony	Interaction
Initial weight (kg)	25.2	25.3	25.2	25.3	0.25	NS	NS	NS
Slaughter weight (kg)	41.1	40.2	40.6	40.3	0.29	NS	*	NS
Live weight gain (g/d)								
<i>initial live weight to 34 kg</i>	303	219	270	319	35.1	NS	NS	*
<i>34 kg to slaughter</i>	195.7	218.3	251.0	222.7	24.11	NS	NS	NS
<i>initial to slaughter</i>	238	212	259	272	17.67	*	NS	NS
Feed intake (kg/d)	1.58	1.56	1.47	1.67	0.061	NS	NS	*
FCE (kg gain/kg DMI)								
<i>initial live weight to 34 kg</i>	0.1937	0.1384	0.1849	0.1897	0.02045	NS	NS	NS
<i>34 kg to slaughter</i>	0.1244	0.1406	0.1726	0.1321	0.01447	NS	NS	*
<i>initial to slaughter</i>	0.1509	0.1409	0.1764	0.1629	0.00774	***	*	NS

<sup>a</sup>SS = slow hourly energy and nitrogen release, SA = slow hourly energy and fast nitrogen release, FS = fast hourly energy and fast nitrogen release, FA = fast hourly energy and slow nitrogen release.

NS = not significant, \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ .

Error degrees of freedom = 15

#### 4.12.4 Blood metabolites

Mean concentration (mmol/l) of plasma urea and  $\beta$ -hydroxy butyrate over a 24 h period are presented in Table 4.13. There was no significant effect of either energy release or synchrony on mean plasma urea concentrations (Table 4.13). However, there was a significant interaction between rate of energy release and synchrony and animals offered diet FS had significantly lower mean plasma urea concentrations than animals offered diet FA (Table 4.13,  $p < 0.05$ ).

Table 4.13. Mean plasma concentrations (mmol/l) of urea and  $\beta$ -hydroxy butyrate (BHB) in ewe lambs fed diets differing in their rates of energy and nitrogen release in the rumen *ad libitum*.

	Diets <sup>†</sup>				s.e.d.	Significance of main effects		
	SS	SA	FS	FA		E	S	I
Urea	5.63	5.12	5.15	5.95	0.367	NS	NS	*
BHB	0.523	0.587	0.363	0.728	0.0965	NS	**	*

<sup>†</sup>SS = slow hourly energy and nitrogen release, SA = slow hourly energy and fast nitrogen release, FS = fast hourly energy and nitrogen release, FA = fast hourly energy and slow nitrogen release, E = energy, S = synchrony, I = interaction.

NS = not significant, \* =  $p < 0.05$ , \*\* =  $p < 0.01$

Error degrees of freedom = 15

There was no significant effect of rate of energy release in the rumen on mean plasma BHB concentrations (Table 4.13). Synchronising the hourly release of energy and nitrogen in the rumen significantly ( $p < 0.01$ ) decreased mean plasma BHB concentrations (Table 4.13). There was a significant interaction between rate of energy release and synchrony and animals offered diet FS had significantly lower mean plasma BHB

concentrations than animals offered diet FA ( $p < 0.05$ , Table 4.13).

The hourly concentrations of plasma urea and BHB are presented in Fig 4.8a and 4.8b respectively. Hourly plasma urea concentrations exhibited a cyclical trend with the highest concentrations for all 4 dietary treatments achieved 2 hours after fresh feed was offered. The rate of release of energy in the rumen did not significantly alter the concentration of urea in plasma of ewe lambs throughout the 24 h period. Synchronising the hourly release of energy and nitrogen in the rumen significantly reduced the plasma urea concentrations 14 h after fresh feed was offered but concentrations were not significantly altered by synchrony at any other sampling time.

Significant interactions between rate of energy release and synchrony occurred 2, 10 and 14 h after fresh food had been offered. Animals offered diet FA had significantly higher ( $p < 0.05$ ) plasma urea concentrations compared to animals offered SA 2 h after feeding. Animals offered diet FS had significantly lower plasma urea concentrations compared to animals offered diet SS at 10 and 14 h after fresh feed was offered. No other significant interactions were observed for plasma urea concentrations.

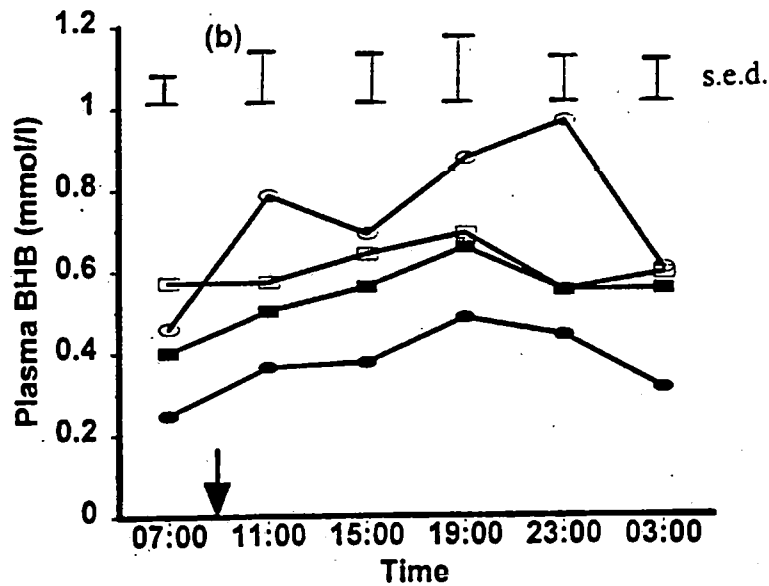
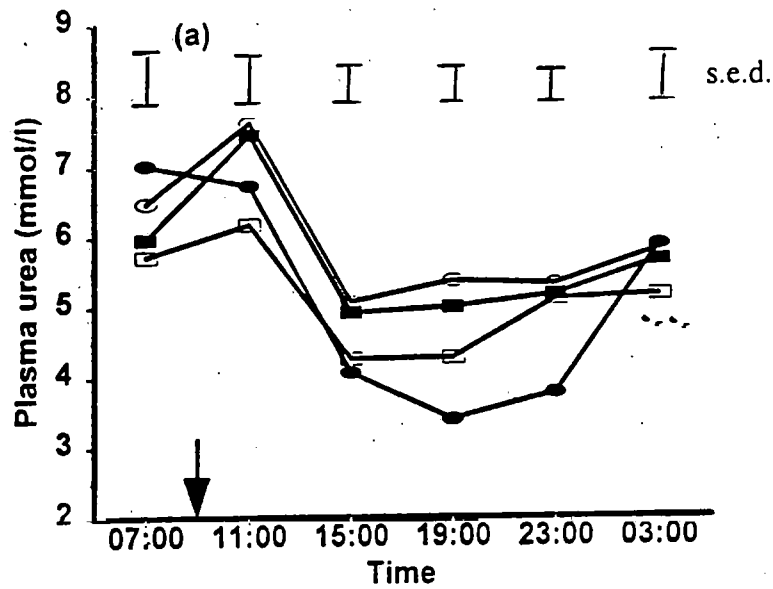


Fig 4.8. Mean hourly plasma concentrations (mmol/l) of (a) urea and (b)  $\beta$ -hydroxy butyrate in ewe lambs offered diets *ad libitum* formulated to have either a slow hourly rumen release of energy and nitrogen (■), a slow release of energy but quick nitrogen (□), a fast release of energy and nitrogen (●) or a fast release of energy but slow release of nitrogen (○). Arrows indicate the time that fresh food was offered.

Mean hourly plasma BHB concentrations tended to increase during the day light hours and then fall during the hours of night for all treatments (Fig 4.8b). Synchronising the rate of release of energy and nitrogen in the rumen significantly lowered hourly concentrations of BHB 2, 6, 14 and 22 h after fresh feed was offered ( $p < 0.05$ ). Similarly, a fast release of energy in the rumen significantly reduced plasma BHB concentrations 2 h before fresh feed was offered ( $p < 0.05$ ). Lambs offered diet FS had significantly lower concentrations of BHB throughout the 24 h period compared to animals offered diet FA.

#### **4.12.5 Carcass components**

Mean values for hot carcass weight, cold carcass weight, killing out proportion, carcass dimensions, *Longissimus Dorsi* area and carcass content of dry matter, ash, fat and nitrogen are presented in Table 4.14. There was no significant ( $p > 0.05$ ) effect of treatment on any of the carcass measurements except killing out proportion, subcutaneous fat depths and *Longissimus Dorsi* area (Table 4.14).



Table 4.14. Carcass components of ewe lambs fed diets differing in their rates of energy and nitrogen release in the rumen *ad libitum*.

	Diets <sup>a</sup>	Significance of main effects						
		SS	SA	FS	FA	s.e.d.	Energy	Synchrony
Hot carcass weight (kg)	19.45	19.88	19.62	19.63	0.327	NS	NS	NS
Cold carcass weight (kg)	18.80	19.18	18.94	18.84	0.273	NS	NS	NS
Killing out proportion	0.4405	0.4615	0.4503	0.4595	0.00815	NS	*	NS
Gigot circumference (cm)	62.70	64.28	65.53	65.15	1.769	NS	NS	NS
Gigot depth (cm)	13.75	14.67	14.67	14.17	0.686	NS	NS	NS
Body length (cm)	54.58	56.00	54.17	52.50	2.311	NS	NS	NS
Chest depth (cm)	26.83	26.42	27.25	26.33	0.661	NS	NS	NS
Kidney fat (g)	390	392	359	313	55.4	NS	NS	NS
Depth of subcutaneous fat (mm)	4.50	5.25	5.33	3.58	0.806	NS	NS	*
<i>Longissimus Dorsi</i> area (cm <sup>2</sup> )	13.43	14.47	13.82	16.80	1.101	NS	*	NS
Carcass dry matter (g/kg)	437.7	448.0	436.3	433.8	18.13	NS	NS	NS
Carcass ash (g/kgDM)	104.7	116.3	108.3	120.7	10.11	NS	NS	NS
Carcass fat (g/kg DM)	517	505	496	496	36.0	NS	NS	NS
Carcass nitrogen (g/kgDM)	58.9	59.0	57.1	61.7	3.89	NS	NS	NS

<sup>a</sup>SS = slow hourly energy and nitrogen release, SA = slow hourly energy and fast nitrogen release, FS = fast hourly energy and nitrogen release, FA = fast hourly energy and slow nitrogen release.

NS = not significant, \* =  $p < 0.05$

Error degrees of freedom = 15

No significant effect of rate of release of energy in the rumen on carcass components was observed (Table 4.14). Synchronising the hourly release of energy and nitrogen in the rumen significantly decreased the killing out proportion of the ewe lambs and significantly reduced the *Longissimus Dorsi* area ( $p < 0.05$ , Table 4.14). There was a significant interaction between rate of energy release and synchrony and lambs fed diet FS had significantly higher depth of subcutaneous fat compared to lambs offered diet FA.

#### **4.12.6 Non carcass composition**

Mean weights of the rumen, intestine, rumen contents and intestine contents of ewe lambs fed synchronous or asynchronous diets are presented in Table 4.15. A fast release of energy in the rumen (diets FS and FA v. SS and SA) significantly increased the amount of digesta in the rumen ( $p < 0.05$ ). Additionally the amount of food present in the lower intestine was significantly reduced by a fast release of energy in the rumen (Table 4.15,  $p < 0.05$ ). An asynchronous or a synchronous supply of energy and nitrogen to the rumen had no significant ( $p > 0.05$ ) effect on non carcass components of ewe lambs (Table 4.15).

Table 4.15. Non carcass components of ewe lambs fed diets differing in their rates of energy and nitrogen release in the rumen *ad libitum*.

	Diets†		Significance of main effects					
	SS	SA	FS	FA	s.e.d.	Energy	Synchrony	Interaction
Rumen (kg)	2.015	2.089	2.112	1.986	0.1524	NS	NS	NS
Intestine (kg)	2.382	2.148	2.119	2.051	0.1390	NS	NS	NS
Rumen contents (kg)	5.00	4.96	5.53	5.08	0.301	*	NS	NS
Intestine contents (kg)	1.799	1.604	1.362	1.437	0.1839	*	NS	NS

†SS = slow hourly energy and nitrogen release, SA = slow hourly energy and fast nitrogen release, FS = fast hourly energy and fast nitrogen release, FA = fast hourly energy and slow nitrogen release.

NS = not significant, \* =  $p < 0.05$

Error degrees of freedom = 15

#### **4.12.7 Rumen metabolism**

Mean rumen fluid pH, total volatile fatty acid concentration (mmol/l) and concentrations (mmol/mol) of acetate, propionate, butyrate, isobutyrate, valerate, iso-valerate and caproate are presented in Table 4.16. The diurnal concentrations of rumen fluid pH and total volatile fatty acids are presented in Fig 4.9 and the diurnal proportions of acetate, propionate and butyrate are shown in Tables 4.17 to 4.20 respectively.

Table 4.16. Influence of a synchronous or asynchronous hourly release of energy and nitrogen in the rumen on mean rumen fluid pH and concentrations of total volatile fatty acids, acetic acid, propionic acid, butyric acid, iso-butyric acid, iso-valeric acid, valeric acid and caproic acid.

	Diets <sup>a</sup>		Significance of main effects					
	SS	SA	FS	FA	s.e.d.	Energy	Synchrony	Interaction
Rumen fluid pH	6.552	6.482	6.345	6.452	0.1095	NS	NS	NS
Total volatile fatty acid (mmol/l)	37.3	44.0	38.1	45.4	7.61	NS	NS	NS
Acetic acid (mmol/mol)	759	761	702	707	22.1	**	NS	NS
Propionic acid (mmol/mol)	175	161	223	220	24.5	**	NS	NS
Butyric acid (mmol/mol)	64	76	72	70	8.9	NS	NS	NS
Iso-butyric acid (mmol/mol)	0.09	0.16	0.21	0.13	0.127	NS	NS	NS
Valeric acid (mmol/mol)	0.82	1.45	1.49	1.76	0.458	NS	NS	NS
Iso-valeric acid (mmol/mol)	0.23	0.25	0.33	0.16	0.158	NS	NS	NS
Caproic acid (mmol/mol)	0.70	1.54	1.14	1.45	0.367	NS	*	NS

<sup>a</sup>SS = slow energy synchronous, SA = slow asynchronous, FS = fast synchronous, FA = fast asynchronous  
NS = not significant, \*\* =  $p < 0.01$ , residual df = 15

#### **4.12.7.1 Rumen fluid pH**

There was no significant effect of rate of release of energy, degree of synchrony or interaction on mean rumen fluid pH (Table 16). The diurnal variation in rumen fluid pH was not significantly altered by the rate of release of energy (Fig 4.9a). Synchronising the rate of release of energy and nitrogen in the rumen significantly increased rumen fluid pH before feeding and 1 h after feeding ( $p < 0.05$ , Fig. 4.9a). Additionally, a synchronous release of energy and nitrogen in the rumen significantly lowered rumen fluid pH 7 h after fresh feed had been offered ( $p < 0.05$ , Fig 4.9a).

At 7 h after feeding there was a significant interaction and animals offered diet FS had significantly lower rumen fluid pH than animals offered diet FA ( $p < 0.01$ ).

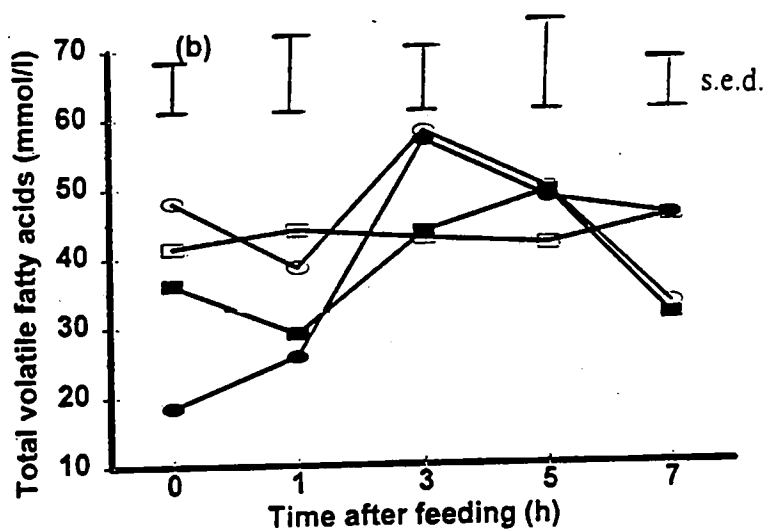
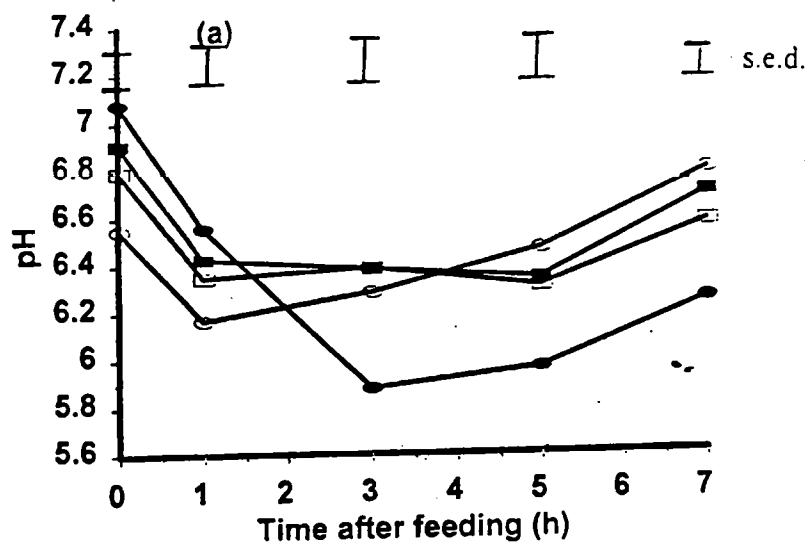


Fig. 4.9. The effect of feeding diets *ad libitum* formulated to have either a slow hourly rumen release of energy and nitrogen (■), a slow release of energy but quick nitrogen (□), a fast release of energy and nitrogen (●) or a fast release of energy but slow release of nitrogen (○) in ewe lambs on (a) rumen fluid pH and (b) total volatile fatty acid concentration.

#### **4.12.7.2 Total volatile fatty acid concentration and proportions of individual volatile fatty acids in rumen fluid**

Mean TVFA concentrations were not significantly affected by rate of release of energy in the rumen, degree of rumen synchronicity or the interaction between rate of energy release and synchrony (Table 4.16). Mean acetate and propionate proportions were significantly increased and decreased respectively by diets promoting a slow release of energy in the rumen (Table 4.16,  $p < 0.01$ ). Synchronising the release of energy and nitrogen in the rumen significantly decreased the mean proportion of caproate in rumen fluid (Table 4.16,  $p < 0.05$ ). No other significant effects were observed on mean rumen fluid VFA concentrations and proportions.

A fast release of energy in the rumen significantly increased the molar proportions of propionate and significantly decreased the proportions of acetate in rumen fluid at all sampling times after fresh feed was offered ( $p < 0.05$ , Tables 4.17 and 4.18).

Diurnal concentrations of TVFA and molar proportions of individual VFA were not significantly altered by the degree of synchronicity except at 0 h after feeding where synchronous rations significantly lowered the concentration of TVFA (27.2 mmol/l v. 44.7 mmol/l, s.e.d. = 4.91,  $p < 0.01$ , Fig 4.9b). Additionally, there was a tendency for animals fed synchronous diets to have higher proportions of propionate and lower proportions of acetate and butyrate after feeding (Tables 4.17 to 4.19).

No significant interactions were observed between rate of energy release and degree of



synchrony for diurnal differences of TVFA or individual proportions of VFA. Although not shown proportions of iso-butyrate, valerate and caproate were not significantly altered by treatment any time after feeding.

Table 4.17. Mean proportion (mmol/mol) of acetate in rumen fluid of ewe lambs fed diets *ad libitum* varying in their degree of rumen synchronicity.

Time after feeding (h)	Diets*				s.e.d.	Significance of main effects		
	SS	SA	FS	FA		E	S	Int
0	747	748	730	719	16.02	NS	NS	NS
1	768	766	714	714	23.76	**	NS	NS
3	761	767	684	677	32.8	**	NS	NS
5	760	768	693	726	27.9	*	NS	NS
7	762	756	689	699	29.9	**	NS	NS

\*SS = slow hourly energy and nitrogen release, SA = slow hourly energy and fast nitrogen release, FS = fast hourly energy and nitrogen release, FA = fast hourly energy and slow nitrogen release offered *ad libitum*. E = energy, S = synchrony, I = interaction.

\*  $p < 0.05$ , \*\*  $p < 0.01$ , error degrees of freedom = 15.

Table 4.18. Mean proportion (mmol/mol) of propionate in rumen fluid of ewe lambs fed diets *ad libitum* varying in their degree of rumen synchronicity.

Time after feeding (h)	Diets <sup>*</sup>				s.e.d.	Significance of main effects		
	SS	SA	FS	FA		E	S	Int
0	184	176	205	212	16.9	*	NS	NS
1	169	159	218	218	23.7	**	NS	NS
3	174	147	238	235	37.3	*	NS	NS
5	172	154	226	202	30.2	*	NS	NS
7	172	161	225	233	32.4	*	NS	NS

<sup>\*</sup>SS = slow hourly energy and nitrogen release, SA = slow hourly energy and fast nitrogen release, FS = fast hourly energy and nitrogen release, FA = fast hourly energy and slow nitrogen release offered *ad libitum*. E = energy, S = synchrony, I = interaction.

\*  $p < 0.05$ , \*\*  $p < 0.01$ , error degrees of freedom = 15.

Table 4.19. Mean proportion (mmol/mol) of butyrate in rumen fluid of ewe lambs fed diets *ad libitum* varying in their degree of rumen synchronicity.

Time after feeding (h)	Diets <sup>*</sup>				s.e.d.	Significance of main effects		
	SS	SA	FS	FA		E	S	Int
0	66	71	64	66	7.8	NS	NS	NS
1	62	71	66	65	9.3	NS	NS	NS
3	63	82	74	84	14.0	NS	NS	NS
5	66	76	78	69	9.5	NS	NS	NS
7	64	80	82	65	12.3	NS	NS	NS

<sup>\*</sup>SS = slow hourly energy and nitrogen release, SA = slow hourly energy and fast nitrogen release, FS = fast hourly energy and nitrogen release, FA = fast hourly energy and slow nitrogen release offered *ad libitum*. E = energy, S = synchrony, I = interaction. Error degrees of freedom = 15.

## 4.13 DISCUSSION

Growing ruminants are generally fed *ad libitum* and it was felt necessary to assess the effect of the diets on lamb performance to determine if daily intake or the hourly pattern of intake would be altered in response to energy source or degree of rumen synchrony. Table 4.20 summarizes the reviews by Forbes (1995), Gill and Romney (1994), Dulphy and Demarquilly (1994) and Forbes and Barrio (1992) on the factors controlling voluntary food intake in ruminants, and indicates the complexity of this subject.

Table 4.20. Various factors thought to be involved with the control of feed intake

Factor	Comments
Volatile fatty acid concentrations	Intra rumen and intravenous infusions of VFA have shown effects of feed intake. Some evidence that different SCFA have different effects
Acetate	Increased concentration reduced intake, some evidence that acetate reduces motility therefore affecting fibre digestion.
Propionate	Very similar effects to acetate intraruminally but much larger effects when infused directly into the hepatic portal vein.
Lactate	Increasing lactate concentrations in the rumen has been accompanied by drastic decreases in intake.
Central nervous system.	Very difficult to ascertain but opiate peptides may be involved in regulating feed intake
Osmotic pressure.	Increases in osmotic pressure are generally accompanied by a decrease in feed intake. Osmotic pressure may increase due to accumulation of end products of fermentation. This may inhibit bacterial hydrolytic capabilities thus reducing passage of material to the abomasum causing a reduction in feed intake. The effects of osmotic pressure are drastically reduced in animals offered clean fresh water <i>ad libitum</i> .
Gastro-intestinal receptors	
(a) mechanical.	Respond to the volume of digesta. Readily found in the rumen, abomasum and duodenum.
(b) chemical.	Sensitive to various chemical concentrations eg VFA found in the rumen, abomasum, duodenum and possibly liver. Nb. Liver control of feed intake may be more concerned with oxidation of fuels rather than detecting circulating concentration of metabolites <i>per se</i> .
Rumen fluid acidity.	Indirect effects on voluntary food intake caused through rumen motility.
Omasum.	The size of the rumen-omasum orifice controls particle exit from the rumen.

Table 4.20 continued.

Factor	Comments
Abomasum.	VFA and lipids reaching the abomasum reduce its motility thereby reducing intestinal passage of nutrients
Duodenum.	See mechanical and chemical receptors.
Glucose and glucose precursors.	Drastic reductions in feed intake due to infusions of propionate into the hepatic vein. Very probable that the ruminant liver is sensitive to its rate of utilization of propionate, controlling intake via the central nervous system.
Growth hormone	Decreases insulin, initiating eating
Insulin	Decreases growth hormone, terminates feeding.
Cholecystokinin (CCK).	Has effects on duodenal motility via contractions.
Secretin.	Decreases intestinal tract outflow rate.
Neuropeptide y.	May increase meal size and frequency.
Melatonin and prolactin.	Associated with day length. Short days have been associated with a shrinking of the rumen in deer hence reducing available capacity.
Feed factors.	
Balance of nutrients.	Particularly important for the rumen microorganisms. Restriction in energy or nitrogen supply may reduce rate and extent of digestion.
Scarcity of diet and relative danger.	Scarce food supplies and threat of predators will reduce intake.
Antinutrients.	Nutrients such as trypsin inhibitors may slow the passage of digesta.
Digestibility.	Effects outflow rate and gut distension.
Palatability.	Rancidity will reduce voluntary feed intake. The physical form of the diet can also effect palatability.
Whole animal factors.	
Breed.	Intestinal tract size may vary between breeds.
Sex.	Intestinal tract size may vary between sex.
Age.	Intestinal tract size may vary between animals of different ages.
Illness.	Animals that are unwell will eat less.
Physiological status	eg. Late pregnancy reduces the capacity of the rumen.

Table 4.20 continued.

Factor	Comments
Sensory factors.	
Taste.	Linked to palatability, generally inclusion of sweet substances such as molasses will increase intake.
Environmental temperature.	In hot environments animals ability to dissipate heat produced by metabolism is reduced and a reduction in VFI is generally observed.

Adapted from Forbes (1995), Gill and Romney (1994), Dulphy and Demarquilly (1994) and Forbes and Barrio (1992).

Table 4.20 indicates that the balance of nutrients could affect voluntary feed intake and Emmans *et al.* (1987) had observed that animals offered diets with a high metabolisable protein to metabolisable energy ratio, would consume greater amounts compared to a balanced diet in order to make up the shortfall in energy. This effect may have been observed in the present experiment between diets supplying a fast rate of energy release in the rumen (FS v. FA). Although growth rates were not significantly different between these two treatments animals offered diet FA consumed significantly more food to achieve the same growth rate. Forbes (1995) implied that overall regulatory control of intake is a combination of both mechanical and chemo-receptors within the body. The intake of animals consuming the slow energy diets (SS and SA) in the current experiment were very similar and the slower rates of organic matter degradability associated with these diets may have involved rumen and duodenum distention as important controls of feed intake (Forbes and Barrio, 1992).

Baile and Meyer (1969) implied that acetate and propionate are major ruminal

intermediary metabolites involved in the metabolic control of voluntary feed intake. However levels of short chain fatty acids found in the present experiment seem to be directly related to the amount of food eaten (ie animals fed asynchronous diets had higher intakes and higher concentrations of volatile fatty acids, see Table 16). In addition, there was no effect on feed intake due to rate of release of rumen degradable energy even though speed of energy release (slow v. fast) significantly increased and decreased proportions of acetate and propionate respectively.

The significantly increased growth rates and feed conversion efficiencies associated with diets supplying a source of fast degradable energy are in direct agreement with Ørskov and MacCloud (1990) and MacRae (1985). These diets (FS and FA) promoted high ratios of glucogenic to ketogenic short chain fatty acids compared to slow release energy diets (SS and SA). The lower feed conversion efficiencies observed could be due to the increased physical work needed to digest the larger proportion of plant cell wall associated with the slow energy diets, as implied by Ørskov and MacCloud (1990). However the diets offered were ground to pass through a 2 mm screen, and this type of physical treatment of neutral detergent fibre has been seen to greatly ease the process of rumen digestion (Beardsley, 1964; Moore, 1964).

The trend to increase voluntary feed intake without a corresponding rise in daily live weight gain in animals offered asynchronous diets resulted in significantly lower feed conversion efficiencies. In direct agreement with the metabolism trial and the previous growth experiment with ram lambs plasma  $\beta$ -hydroxy butyrate were significantly increased due to asynchrony even though rumen concentrations of butyrate were

unaffected. These results give further evidence to support the hypothesis that asynchronous diets were deficient in glucose precursors.

Contrary to the previous growth experiment the pattern of growth followed during the present experiment displayed no signs of compensatory growth after 34 kg live weight had been reached, apart from animals fed diet SA. The majority of carcass components displayed no differences due to dietary treatment although the trend for the intestine weight to be heavier in lambs offered synchronous diets may be an indication of differences in energy partitioning between gut and whole body metabolism (Johnson *et al.*, 1990). This increase in intestine weight and the lack of differences in carcass weight resulted in animals fed synchronous diets having significantly lower killing out proportions compared with those fed asynchronous diets. The increase in *L. Dorsi* area and increase in killing out percentage in lambs fed asynchronous diets indicate that although feed conversion efficiency was increased by synchrony this was not translated into more edible material when diets were offered *ad libitum*.

#### **4.14 CONCLUSIONS**

*Ad libitum* access to diets formulated to be synchronous for their hourly release of energy and nitrogen in the rumen significantly improved feed conversion efficiency.

The increase in feed conversion efficiency associated with synchronous diets did not result in an increase in carcass weight or protein content.



## **CHAPTER 5**

### **5.0 EFFECTS OF SYNCHRONISING THE HOURLY RELEASE OF ENERGY AND NITROGEN IN THE RUMEN IN DIETS WITH SIMILAR CARBOHYDRATE SOURCES ON LACTATION AND FEED INTAKE OF FRIESLAND EWES**

#### **5.1 Introduction**

Ensilage of grass exposes the nitrogenous content to extensive degradation by proteases resulting in high levels of non protein nitrogen (amino acids, amines, ammonia) which are soluble in rumen liquor (McDonald *et al.* 1988). Conversely the fermentable organic matter content of grass silage is primarily composed of plant cell wall which has a slower rate of degradation and contains less immediately soluble material. Ensilage of grass, therefore, produces a feedstuff that is particularly asynchronous for the hourly supply of nitrogen and organic matter to rumen microbes. Work to evaluate the effects of pattern of nutrient release from grass silage on microbial metabolism (e.g. Rooke *et al.* 1987) has indicated low microbial efficiencies when grass silage was offered as the sole feed. Subsequent improvements have been observed by the provision of soluble sugars.

Formulation of diets that are synchronous for their hourly energy and nitrogen release in the rumen has been shown to increase the efficiency of microbial protein synthesis (Sinclair *et al.* 1995) and results described in the present work have shown improved feed

conversion efficiencies of lambs fed synchronous diets either at a restricted level or *ad libitum* (Chapter 4).

The results from Chapter 4 demonstrated that different carbohydrates produce different rumen volatile fatty acid ratios in the rumen. Different proportions of glycolytic to ketogenic precursors have been shown to alter the partitioning of nutrients in the lactating ruminant (Webster, 1987) and can result in differences in milk yield and composition (Webster, 1987). For this reason the diets in the present experiment were formulated to have a similar carbohydrate composition and be similar in their rates of release of organic matter in the rumen. The objectives of the present experiment were:

1. the formulation of diets, based on grass silage, to be either synchronous or asynchronous for their hourly release of nitrogen and energy in the rumen but to have similar rates of organic matter release
2. to assess the effects of hourly rumen synchronicity on milk production, feed intake and rumen and whole body metabolism of milking ewes.

## **5.2 Experiment 5a. *In situ* characterisation of grass silage**

### **5.2.1 Animals and basal diet**

Four wether sheep aged 2 years, weighing c. 65 kg and fitted with permanent rumen cannulae (37 mm diameter), were housed in individual slatted floor pens with free access to water and mineral licks and kept under continuous lighting. A basal diet containing (g/kgDM) 400 silage, 500 barley, 75 soya and 25 vitamins and minerals was offered *ad libitum* with fresh food made available daily at 0900 h. Refusals were discarded every second day.

### **5.2.2. Silage preparation and incubation procedure**

Fresh samples of silage were chopped to a length of approximately 2 cm and the incubation procedure was carried out as described in section 3.2.1. Degradability coefficients and the hourly degradation was calculated as is described in sections 3.2.2 and 3.5 respectively.

## **5.3 RESULTS**

Proximate analysis and fibre content of the silage is presented in Chapter 3, Table 3.1 and degradability coefficients for organic matter and nitrogen are given in Table 5.1. The immediately soluble fraction (a) was approximately 75% of the nitrogen content of the grass silage. Conversely only 35% of the organic matter content was immediately soluble

(Table 5.1). Neither the organic matter nor the nitrogen content of the grass silage incorporated a lag phase.

Table 5.1. Degradability coefficients for organic matter and nitrogen for silage.

	a	b	c	lag	r <sup>2</sup>	a+b
Organic matter	0.3541	0.3704	0.0400	0.000	0.980	0.7245
Nitrogen	0.7577	0.1213	0.0669	0.000	0.8790	0.8790

## 5.4 DISCUSSION

The characterisation procedure has already been extensively discussed in the current work (see section 3.7).

When grass is ensiled a large proportion of the protein content is attacked by proteases converting much of it to non protein nitrogen. Once ingested these NPN sources, such as ammonia, amino acids and amines are rapidly solubilised and are therefore, in theory, immediately available to the rumen microorganisms for metabolism. Conversely, much of the carbohydrate fraction associated with grass silage is of a fibrous nature, and is therefore much more slowly degraded. This pattern of release of energy and nitrogen in the rumen was observed in the present study. Over 70% of the nitrogen content of the grass silage analysed was estimated to be contained in the immediately soluble fraction (Table 5.1) but only 35% of the organic matter content. This mismatch of supply of energy and nitrogen confirms grass silage to be a particularly asynchronous type diet (Rooke *et al.* 1987).

Presentation of feed material in the dacron bag should mimic the effects of mastication and rumination as these are mechanical methods of digestion imposed by the host which are not utilised for feed contained in a dacron bag. To reproduce the effects of chewing fresh forage material, Doreau and Ould-Bah (1992) concluded that the best method of preparation was by coarse chopping as this exposed a similar surface area for microbial colonisation as mastication. For the present study silage was chopped before incubation and characterisation took place.

Choice of the basal ration and the level at which it is fed during the characterisation procedure may effect the degradability figures produced (Weakley *et al.* 1983; Lindberg, 1981; Leng and Nolan, 1984). In the present study a ration of 60:40 for concentrate: forage (DM basis) was offered *ad libitum* so that similar rumen environments would be attained during the characterisation procedure and subsequent lactation experiment. It has been suggested (Weakley *et al.* 1983) that basal rations based on silages may promote bacterial slime which may block the pores of the bags, preventing escape or access of microorganisms. During the present characterisation procedure it was noted that the bags were slimy after retrieval, particularly after the longer incubation times although the effect of this contamination on the degradability figures calculated is difficult to assess.

### **5.5 Experiment 5b, Formulation of experimental diets**

Diets (Table 5.1) were formulated using the S.I.R.E. computer program (section 3.6.1) containing the degradability coefficients measured in experiment 1. All diets contained a ratio of 60:40 concentrate to silage (dry matter basis) and were fed as a complete feed. Two diets (synchronous and asynchronous) were formulated to have similar patterns of organic matter release in the rumen but to differ in the rate of nitrogen release, as indicated by the synchrony index (Table 5.3). A third diet (intermediate) was formulated using 50% of each ingredient present in the other 2 diets (Table 5.2). Diets were formulated to have similar predicted contents of metabolisable energy (MJME/kgDM) and (g/kgDM) metabolisable protein, digestible undegradable protein, neutral detergent fibre, hemi-cellulose, cellulose and starch (Table 5.2). Because the hourly pattern of food intake could not be accurately predicted the rations were formulated assuming that the

entire day's consumption of food would occur in the first hour after fresh feed had been offered.

The ratio of N to OMTDR assumed to be the most efficient for the production of rumen microorganisms was 28 gN/kg OMTDR.



Table 5.2. Dietary composition and predicted content of metabolisable energy (ME), crude protein, fermentable metabolisable energy (FME), effective rumen degradable protein (ERDP): fermentable metabolisable energy, neutral detergent fibre (NDF) and starch content of 3 diets. (Rumen outflow rate was assumed to be 0.06/h (see section 5.5.1 for explanation of assumed rumen outflow used)).

	Diets		
	Synchronous	Intermediate	Asynchronous
Dietary composition			
Silage	400	400	400
Wheat straw	50	55	60
Barley	90	75	60
Malt distillers dark grains	20	75	130
Fish meal	30	41	52
Sugar beet pulp	40	57.5	75
Tapioca	215	192.5	170
Rape seed meal	120	60	---
Urea	---	1.5	3
Fat	10	17.5	25
Vitamins/minerals	25	25	25
ME (MJ/kgDM)	11.8	11.8	11.9
Crude protein (g/kgDM)	164	167	170
FME (MJ/kgDM)	9.78	9.65	9.51
Metabolisable protein (g/kgDM)	102	102	102
Digestible undegradable protein (g/kgDM)	40.5	39.3	38.0
NDF (g/kgDM)	360	366	371
Starch (g/kgDM)	189	167	144
ERDP/FME	9.9	10.4	10.9

Raw materials were chosen primarily for their degradability characteristics (Tables 3.2, 3.3 and 5.1) but also to supply the host with a similar predicted amount of metabolisable energy and protein (Table 5.2). Predicted supply of energy and protein to both the host and the rumen was calculated as AFRC (1990) and AFRC (1992) respectively. All diets

contained (on a dry matter basis) 400 g/kg silage and 25 g/kg of a multi-mineral supplement (Ewe minerals, Trouw Nutrition). The levels of inclusion of energy sources (winter wheat straw, barley, sugar beet feed and tapioca) were similar between treatments. In order to achieve either a synchronous or asynchronous supply of energy and nitrogen to the rumen, protein source (malt distillers dark grains, fishmeal, rapeseed meal and urea) differed between treatments (Table 5.2). The predicted supply of metabolisable energy and fermentable metabolisable energy (MJ/kgDM) and (g/kgDM) crude protein, metabolisable protein digestible undegradable protein, plant cell wall and starch were similar for all 3 diets (Table 5.2).

The ingredients, excluding the silage, were ground through a 2 mm screen and mixed in a commercial mobile mixer. The concentrate was mixed in 1 tonne batches. The concentrate and silage portions of the complete diet were mixed daily by hand to achieve a concentrate to silage ratio of 60:40 (dry matter basis).

The extent of degradability of organic matter and nitrogen on a daily basis and the predicted release of organic matter and nitrogen for each h over a 24 h period are given in Table 5.3 and Fig 5.1 respectively.

Table 5.3. Predicted daily extent of organic matter and nitrogen degraded in the rumen of 3 diets. Calculations are based on a rumen outflow rate of 6% per h.

	Diets		
	Synchronous	Intermediate	Asynchronous
Organic matter degraded (g/d)	513.4	503.2	493.0
Nitrogen degraded (g/d)	18.0	19.1	20.1
Organic matter degradability	0.58	0.58	0.57
Nitrogen degradability	0.69	0.72	0.74
Daily ratio of nitrogen:organic matter degraded in the rumen	35	38	41
Synchrony index <sup>†</sup>	0.88	0.75	0.61

<sup>†</sup>Calculated as described in section 3.5. The optimum ratio of gN/kgOMTDR is assumed to be 28 for the lactating ruminant

Figures are based on 1000 g dry matter offered once daily.

The predicted amounts of organic matter and nitrogen degraded over a 24 h period (Table 5.3) were similar for all 3 diets. In addition the daily ratio of gN/kgOMTDR is similar for the 3 treatments at 35 gN/kgOMTDR for the synchronous and 41 gN/kgOMTDR for the asynchronous diets. The predicted amount of organic matter degraded each hour after feeding (Fig 5.1) was very similar for all treatments. In contrast, the predicted pattern of release of nitrogen (Fig 5.1) showed a higher peak for the asynchronous diet compared to the synchronous diet immediately after consumption.

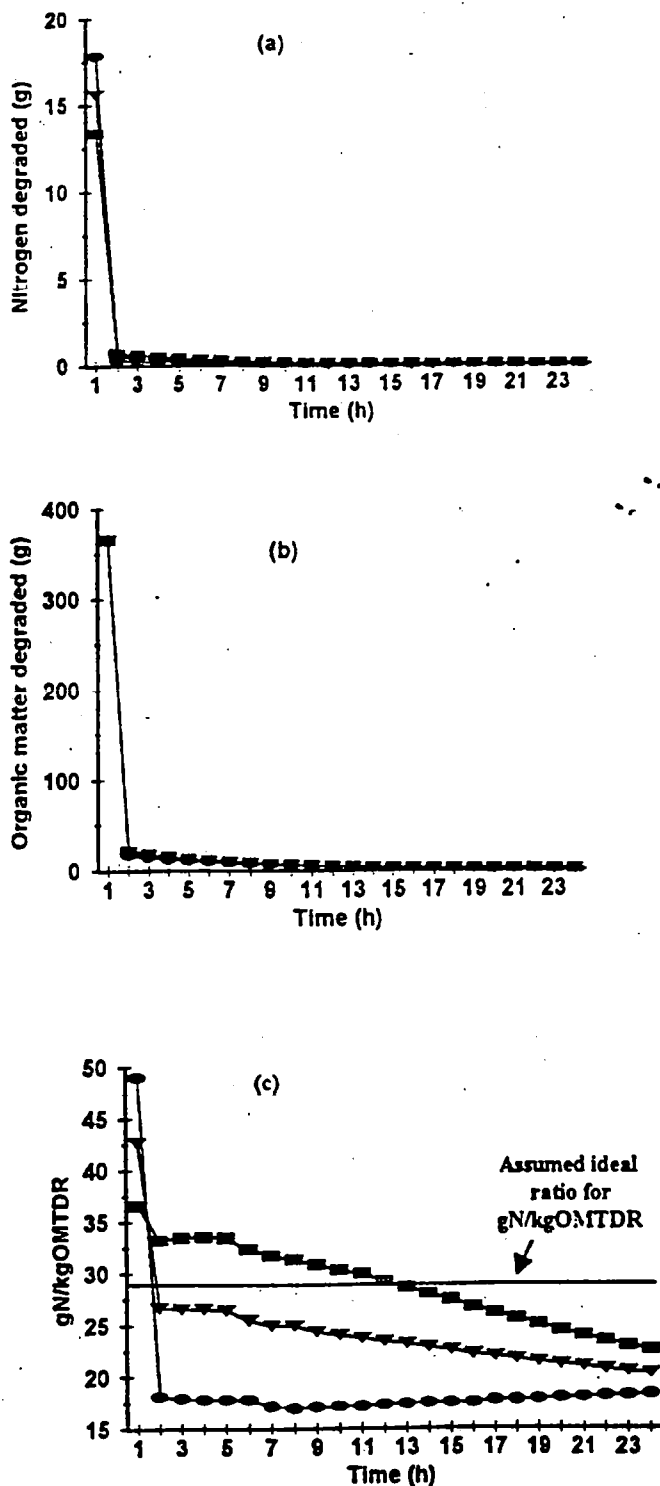


Fig. 5.1. Predicted rates of release of (a) nitrogen and (b) organic matter and (c) predicted ratio of g nitrogen degraded per kg of organic matter truly degraded in the rumen for diets formulated to be either synchronous for their hourly release of energy and nitrogen (■), intermediate for the hourly release of energy and nitrogen (▼) or asynchronous for the hourly supply of energy and nitrogen in the rumen (●). Calculations are based on a total of 1000 g of dry matter eaten in the first hour and a rumen outflow rate of 0.06/h.

### **5.5.1 Level of feeding and nutrient concentration of experimental feeds**

Diets were offered *ad libitum* (115 % of achieved intake) daily at 0730 h. Refusals were weighed and then discarded every other day. The weight of the refusals were used to calculate the new amount of feed required. Previous work (Sinclair and Scoffield, 1996) had indicated that the mean dry matter intake of Friesland ewes was approximately 0.04 times live weight and ewes fed a ration based on 60:40 dry matter basis of concentrate to silage had a rumen outflow rate of approximately 0.06 per hour (Sinclair and Scoffield, 1996). The equations supplied by AFRC (1990) and AFRC (1992) are for ewes with lambs at foot and it is unclear how these requirements relate to milking sheep. Energy and protein concentration of the diets (Table 5.2) to meet ewes requirements were calculated using an average live weight of 70 kg with an average milk yield of 3 kg per day having a composition of 70 g/kg fat and 48.9 g/kg protein. Initial requirements were also calculated for ewes that were 28 days into lactation. In addition energy requirements were increased by allowing for an increase of body weight of 0.05 kg per day in order for the ewes the potential of increasing in body condition score. The metabolizability of the ration (ME/GE) was assumed to be 0.6.

### **5.6 Animals**

Twenty four multiparous Friesland ewes weighing c. 70 kg were housed individually, bedded on sawdust and kept under continuous lighting with free access to water. Wool was sheared from the necks of each ewe fortnightly to aid with blood sampling.

### **5.6.1 Pre-experiment management**

Ewes were group housed according to the number of lambs expected and moved to individual pens 2 days prior to expected lambing. All ewes lambd over a period of 7 d and lambs were removed 2 days after lambing. Milking of the ewes commenced immediately and the ewes were milked twice daily at 08.00 and 16.00 h. Once lambing was complete, diets were changed from a hay plus concentrate (BOCM ewe nuts, 16% protein) ration to a silage plus concentrate mix in order to adapt the ewes to the silage based diet. Silage was offered *ad libitum* and concentrates (BOCM ewe nuts, 16% protein) were added to the silage and mixed by hand 3 times a day (0.5 kg per feed) to minimise the risk of acidosis.

## **5.7 Collection and analysis**

The experiment comprised of 3 periods with each period being of 4 weeks duration. Each ewe was allowed 2 weeks adaptation to experimental diets before the collection period commenced.

### **5.7.1 Feeds**

Weekly sub-samples of the rations were taken during the collection periods, dried to a constant weight, pooled and then analysed for organic matter, crude protein, neutral detergent fibre, acid detergent fibre, acid detergent lignin and ether extract (section 2.1).

### 5.7.2 Milk collection

Total milk yield was recorded 4 times during each collection period and was also split into morning and afternoon milking. The days of sampling were Tuesday and Thursday afternoons and Wednesday and Friday mornings (see Table 5.4). Milk samples (50 ml per recording) were taken from each ewe and immediately stored at  $-20^{\circ}\text{C}$  prior to analysis.

Table 5.4. Schedule of collection periods for ewes fed diets differing in their degree of synchrony in the rumen.

Week	Day	Procedure
3	Mon	
	Tue	Blood sampling at 0600 and 1800 h, milk record (pm).
	Wed	milk record (am)
	Thu	Blood sampling at 1000 and 2200 h, milk record (pm)
	Fri	milk record (am), blood sampling at 1400 and 0200 h.
4	Mon	
	Tue	Milk record (pm)
	Wed	Milk record (am), pattern of intake (6 ewes)
	Thu	Pattern of intake (6 ewes), milk record (pm)
	Fri	Milk record (am), rumen sampling at 0,1,3,5,7 h after feeding.

### **5.7.3 Milk analysis**

Milk samples were analysed for water, total fat, total protein and lactose content (g/kg) using a Milkoscan 103 automated milk analyser after appropriate calibration with high fat samples. Samples were allowed to defrost at room temperature and then heated to 40°C in a circulating water bath. Samples were homogenized for 30 seconds and then a sub-sample (1 ml) injected into the milk analyser. Both the homogenizer and sampling probe were washed with warm (40°C) deionised water between samples.

### **5.7.4 Blood collection**

Blood samples (7 ml) were taken into both heparin and potassium oxalate blood tubes (vacutainer) from the jugular vein by venepuncture every 4 h for a 24 h period. In an attempt to cause minimum effect on feed intake blood sampling was undertaken over 3 days, two samples per day (See Table 5.4).

### **5.7.5 Blood analysis**

Blood samples were processed and analysed as described in section 4.5.4.7.

### **5.7.6 Pattern of feed intake**

Individual feed troughs were mounted on Avery balances (model No. 3359) which had been modified to have an additional voltage output which was connected to a digital



logger (Grants, Squirrel 1250). The logger was programed to record voltage output every 5 mins for a 24 h period. Due to the number of balances available and the space between pens for setting up the balances and logger, patterns of intakes of the ewes were measured over 2 days (see Table 5.4). A total of 6 ewes (2 ewes per treatment) were measured on each day allowing 30 mins change over time between the consecutive 24 h periods.

#### **5.7.7 Rumen fluid collection**

Samples of rumen fluid were taken using a stomach tube 0, 1, 3, 5 and 7 h after the morning feed on the last day of the collection period (see Table 5.4). Samples were processed and analysed as described in section 2.1.12.

#### **5.7.8 Statistical analysis**

All results were subjected to a analysis of variance procedure as a Latin square design with 3 periods using GENSTAT 5.1 (Lawes Agricultural Trust, 1990). Treatment means were compared by the least significant difference method protected by a significant F-value.

### **5.8 RESULTS**

The chemical analysis of the 3 diets is presented in Table 5.5. The 3 diets were similar in their chemical composition. The content of crude protein was approximately 15 g/kgDM below predicted values but was similar between treatments.

**Table 5.5. Mean crude protein, ether extract, organic matter, neutral detergent fibre, acid detergent fibre and acid detergent lignin content of three diets formulated to differ in their rate of N and OM release in the rumen fed to lactating ewes (all units are g/kgDM).**

	Diet		
	Synchronous	Intermediate	Asynchronous
Crude Protein	151	155	155
Ether Extract	29	40	50
Organic Matter	907	901	907
NDF	321	333	355
Hemicellulose	119	126	132
Cellulose	156	181	186

Mean milk yield and composition and mean daily feed intakes are presented in Table 5.6.

There was a tendency for animals fed synchronous diets to have a higher milk yield compared to animals receiving the asynchronous or intermediate diets although this difference was not significant. There was no significant difference between treatments in milk protein content. However, ewes fed the synchronous diet tended to have a higher yield (g/d) of protein compared to those offered the asynchronous diet ( $p=0.05$ ). Ewes fed the asynchronous diet had significantly higher milk butterfat percentages than those offered either of the other two treatments ( $p<0.001$ ). Dry matter intakes (Table 5.6) did not differ significantly between treatments.

Table 5.6. Mean yield of milk, butterfat, protein and lactose and mean milk concentration of butterfat, protein and lactose and mean dry matter intakes of ewes fed diets varying in their degree of rate of release of energy and nitrogen in the rumen.

	Diet			s.e.d.
	Synchronous	Intermediate	Asynchronous	
Yield (g/d)	1929	1807	1811	77.8
Butterfat (%)	5.195 <sup>a</sup>	5.582 <sup>b</sup>	5.743 <sup>b</sup>	0.1433
Protein (%)	4.680	4.630	4.584	0.0451
Lactose (%)	4.929	4.918	5.113	0.1903
Butterfat (g/d)	99.2	99.1	100.5	4.00
Protein (g/d)	89.9	82.7	82.4	3.37
Lactose (g/d)	95.1	89.0	93.0	5.05
Dry matter intake (kg/d)	4.55	4.71	4.57	0.915

Means in the same row with different superscripts are significantly different ( $p < 0.001$ ) residual degrees of freedom = 44 (see appendix 1).

### 5.8.1 Blood metabolites

Mean plasma concentrations (mmol/l) of urea and  $\beta$ -hydroxy butyrate are presented in Table 5.7. Altering the degree of rumen synchrony did not significantly affect the mean concentration of plasma  $\beta$ -hydroxy butyrate (Table 5.7). There was a trend for animals fed diets increasing in their degree of synchronicity to have an increased concentration of  $\beta$ -hydroxy butyrate (Table 5.7). Mean plasma urea concentrations were significantly lower in animals fed the synchronous diets compared to those receiving either the intermediate or asynchronous diets ( $p < 0.001$ , Table 5.7).

Table 5.7. Mean plasma concentrations (mmol/l) of urea and  $\beta$ -hydroxy butyrate in ewes fed diets differing in their rate of release of energy and nitrogen in the rumen.

	Diets			s.e.d.
	Synchronous	intermediate	Asynchronous	
$\beta$ -hydroxy butyrate	0.678	0.619	0.599	0.0366
Urea	6.539 <sup>a</sup>	7.417 <sup>b</sup>	7.750 <sup>b</sup>	0.2082

Means with differing superscripts are significantly different ( $p < 0.001$ )  
residual degrees of freedom = 8 (see appendix 2)

Hourly plasma  $\beta$ -hydroxy butyrate concentrations followed a similar cyclical pattern throughout the 24 h period for animals fed synchronous, intermediate or asynchronous diets (Fig 5.2a). Concentrations of  $\beta$ -hydroxy butyrate tended to be higher during daylight hours compared to night whilst animals receiving the synchronous supply of energy and nitrogen to the rumen had significantly elevated concentrations of plasma  $\beta$ -hydroxy butyrate compared to animals fed either the intermediate or the asynchronous diets at 14.00 h and 18.00 h ( $p < 0.05$ ).

Hourly plasma urea concentrations over the 24 h period are shown in Fig 5.2b. Plasma urea concentrations were consistently lower for animals fed the synchronous diets compared to both the intermediate and asynchronous treatments. These were significant at 1000 h ( $p < 0.01$ ), 1400, 1800 and 2200 h ( $p < 0.001$ ) 02.00 h ( $p < 0.05$ ) compared to animals fed the asynchronous ration, and at 1000 h ( $p < 0.01$ ), 1400 h ( $p < 0.001$ ), 1800 h ( $p < 0.05$ ) and 2200 h ( $p < 0.001$ ) compared to animals fed the intermediate ration.

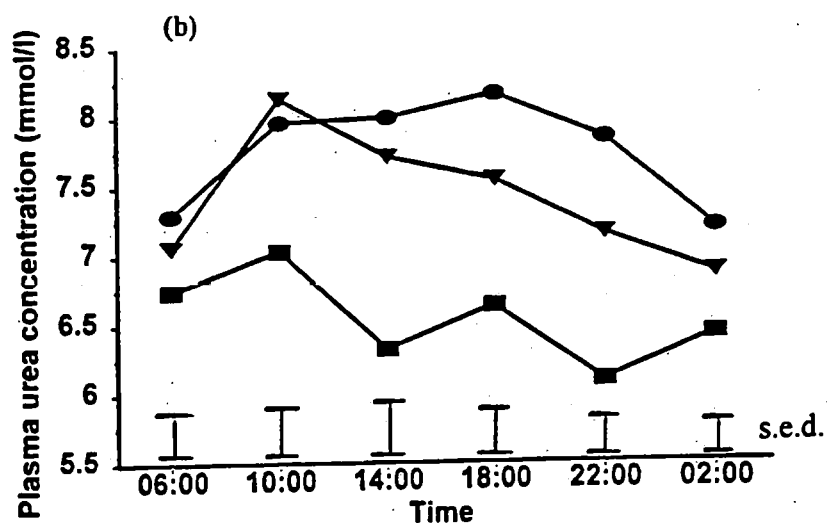
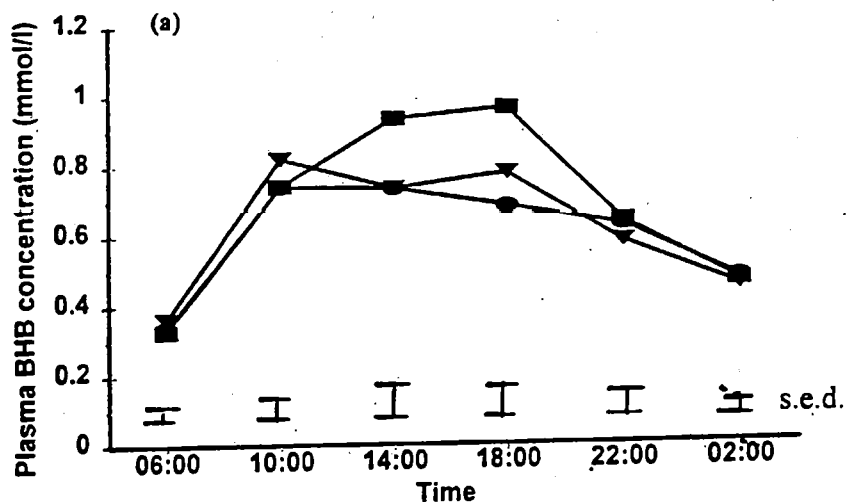


Fig. 5.2. Mean plasma  $\beta$ -hydroxy butyrate (a) and urea (b) concentrations (mmol/l) in ewes fed diets formulated to be either synchronous for their hourly release of energy and nitrogen (■), intermediate for the hourly release of energy and nitrogen (▼) or asynchronous for the hourly supply of energy and nitrogen in the rumen (●). Ewes were offered fresh food at 09.00h.

### 5.8.2 Rumen pH and volatile fatty acid concentrations

Mean rumen pH and concentrations of total volatile fatty acids (TVFA), acetate, propionate, butyrate, isobutyrate, valerate, isovalerate and caproate are presented in Table 5.8. Diurnal variation of total VFA and rumen fluid pH presented in Tables 5.9 and 5.10 respectively.

Table 5.8. Mean rumen fluid pH, concentrations of total VFA and the molar proportions of individual VFA from ewes fed diets differing in the rate of energy and nitrogen degradation on the rumen.

	Diets <sup>#</sup>			s.e.d.
	Syn	Int	Asy	
pH	6.42	6.40	6.55	0.055
Total VFA (mmol/l)	58.3	66.2	59.3	6.37
Acetate (mmol/mol)	741	725	739	8.7
Propionate (mmol/mol)	187	201	193	9.6
Butyrate (mmol/mol)	66	67	61	6.1
Isobutyrate (mmol/mol)	1.25	1.43	1.42	0.078
Valerate (mmol/mol)	1.90	2.06	2.01	0.138
Isovalerate (mmol/mol)	1.59	1.95	2.06	0.247
Caproate (mmol/mol)	1.44	1.45	1.35	0.146

<sup>#</sup>Syn = synchronous release of organic matter and nitrogen in the rumen, Int = intermediate synchronicity, Asy = asynchronous rations.

Residual degrees of freedom = 8 (see appendix 2).

No significant differences were observed between treatments for mean daily rumen fluid pH, total concentration or individual molar proportions of volatile fatty acids (Table 5.8).

Ewes fed the synchronous rations had significantly lower concentrations of total VFA in

rumen fluid before fresh feed was offered ( $p < 0.05$ , Table 5.9). No other significant differences were observed in diurnal concentrations of TVFA. Rumen fluid pH was significantly lower at 5 h after feeding in ewes fed the synchronous diets. There was no other significant effect of diet on rumen fluid pH ( $p < 0.05$ , Table 5.10).

Table 5.9. Mean rumen fluid concentrations of total volatile fatty acids (mmol/l) in ewes fed complete diets formulated to have similar rumen carbohydrate release but differ in the pattern of nitrogen degradation.

Time after feeding (h)	<u>Diets</u> Syn	Int	Asy	s.e.d.
0	36.8 <sup>b</sup>	68.9 <sup>a</sup>	51.3	8.48
1	63.3	65.2	55.2	6.86
3	59.7	56.2	62.6	6.01
5	64.4	69.4	62.8	6.75
7	67.2	71.2	64.3	7.33

residual degrees of freedom = 8 (see appendix 2), means in the same row with different superscripts are significantly different ( $p < 0.05$ ).

Table 5.10. Mean rumen fluid pH in ewes fed complete diets formulated to have similar rumen carbohydrate release but differ in the pattern of nitrogen degradation.

Time after feeding (h)	<u>Diets</u> Syn	Int	Asy	s.e.d.
0	6.89	6.72	8.83	0.071
1	6.50	6.52	6.56	0.072
3	6.36	6.35	6.54	0.108
5	6.16	6.18	6.43	0.077
7	6.21	6.24	6.37	0.134

residual degrees of freedom = 8 (see appendix 2).

### 5.8.3 Pattern of intake

Dry matter intake (g/h), proportion of dry matter intake (g/100g/h) and cumulative intake (gDM) are presented in Figs 5.3a, 5.3b and 5.3c respectively. There was a trend for animals offered diets with an increasing degree of synchronicity to consume more feed in the first h after fresh food had been offered (Fig 5.3a) and also to achieve higher cumulative intakes over the 24 h period (Fig 5.3c) although neither were significant. There were no significant differences in the proportion of food consumed each hour (Fig 5.3b), except at hours 6 and 15 when animals fed synchronous diets ate proportionately less feed than animals offered asynchronous diets ( $p < 0.05$ ). Dry matter intake (g/4h) and proportion of dry matter intake (g/g/4h) are presented in Tables 5.11 and 5.12 respectively and showed no significant differences between treatments. However there was a trend that animals offered diets with increasing synchronicity (synchronous and intermediate rations) consumed more food (gDM) and a larger proportion of the total daily intake (g/g) during the first 4 h after fresh food had been offered although neither of these observations were significant ( $p > 0.05$ , Tables 5.11 and 5.12).



Table 5.11. Dry matter intake (g per 4 h) of lactating Friesland ewes of diets of differing degree of rumen synchronicity offered *ad libitum*.

Hours	Diets			s.e.d.
	Syn	Int	Asy	
1-4	1777	1729	1455	243.0
5-8	1143	1126	1085	147.7
9-12	1019	918	944	104.3
13-16	472	504	489	79.7
17-20	112	148	85	48.6
21-24	116	55	87	41.0

residual degrees of freedom = 20 (see appendix 3).

Table 5.12. Proportion of dry matter intake (g/100g per 4 h) of lactating Friesland of diets of differing degree of rumen synchronicity offered *ad libitum*.

Hours	Diets			s.e.d.
	Syn	Int	Asy	
1-4	35.8	35.7	35.1	3.77
5-8	25.9	25.4	26.3	2.96
9-12	23.2	21.8	23.1	2.69
13-16	10.2	12.2	11.5	1.54
17-20	2.3	3.9	2.0	1.06
21-24	2.6	1.0	2.1	0.84

residual degrees of freedom = 20 (see appendix 3).

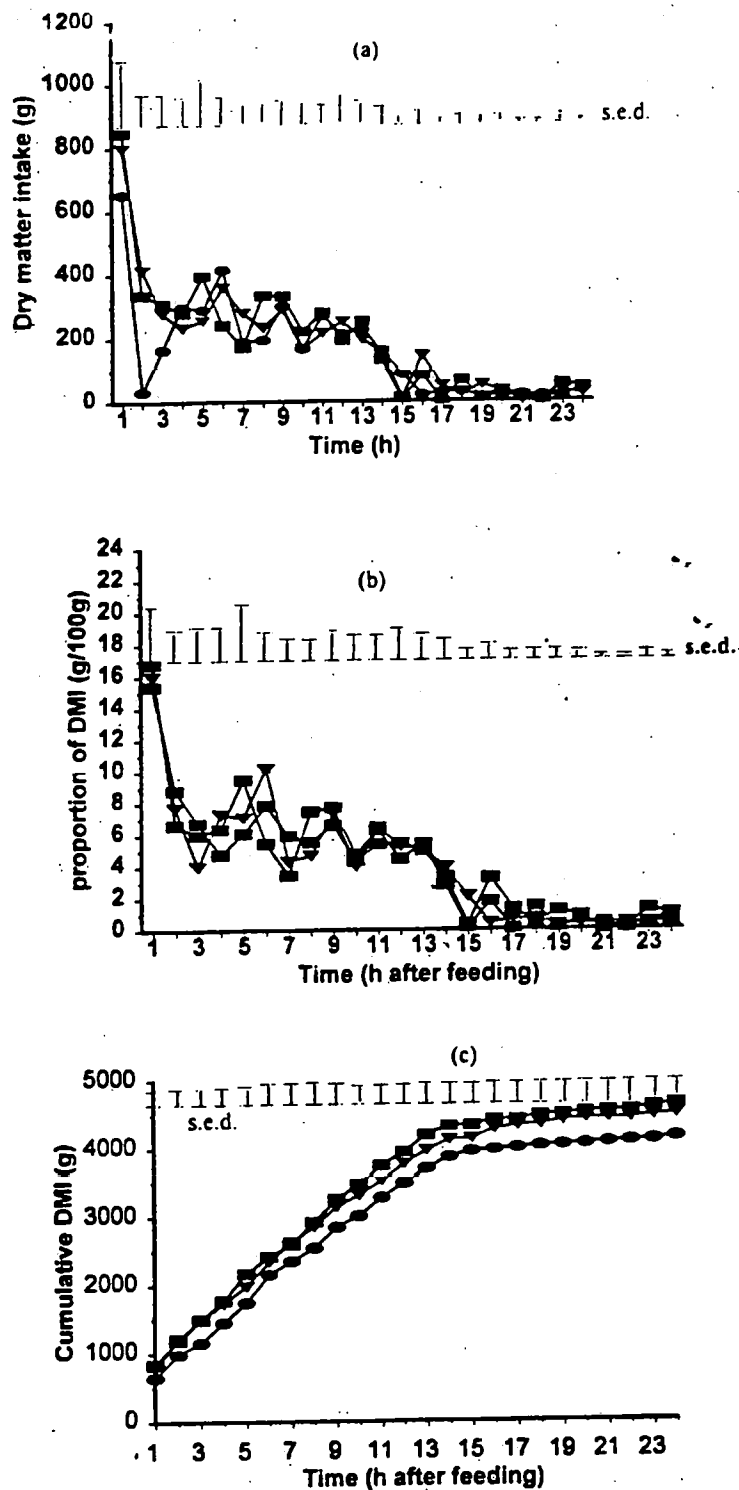


Fig 5.3. (a) Mean dry matter intakes (g/h), (b) mean hourly proportion of dry matter intake (g/100g/h) and (c) mean cumulative dry matter intake (g) of milking sheep fed diets formulated to be either synchronous for the hourly release of energy and nitrogen in the rumen (■), intermediate for the hourly release of energy and nitrogen (▼) or asynchronous (●).

## 5.9 DISCUSSION

Similar to the previous experiments reported in this thesis synchronous and asynchronous diets were formulated by altering the ingredient composition of the ration. However to avoid differences in nutrient partitioning of nutrients between body and milk associated with different volatile fatty acids produced in the rumen (Webster, 1987; Sutton, 1981) carbohydrate sources and therefore predicted rates of carbohydrate breakdown were kept similar between diets. Previous experiments (Casper and Schingoethe, 1989; Herrera-Saldana and Huber, 1989; Aldrich *et al.*, 1993) assessed varying rates of energy and protein release in the rumen using factorial approaches containing fast and slow energy sources. The present experiment differs from these methods of appraisal in that incremental degrees of synchrony were used (synchronous, intermediate and asynchronous) making it possible to assess any trends.

Similarly to the experiments described in chapter 4, the rations formulated for the present experiment were predicted to supply similar levels of metabolisable nutrients to the host according to current formulation techniques (AFRC, 1992). To achieve differences in the degree of synchrony it was found necessary to include greater amounts of urea with decreasing synchrony. However the amount used was extremely small (maximum of 0.3%). Additionally, the inclusion of fish meal, a high quality protein (Chancellor, 1983) increased with increasing asynchrony.

The chemical analysis of the diets (Table 5.3) was similar to the predicted composition (Table 5.1). It is therefore reasonable to assume that the predicted pattern of nutrient

release would also have been similar as presented in Table 5.2 and Fig. 5.1. Chamberlain *et al.* (1993) indicated differences in microbial efficiency due to different rates of fermentation of carbohydrates. The predicted pattern of fermentation of organic matter in the present study (Fig 5.1b) shows there to be no appreciable differences between the diets.

A proposed beneficial effect of a synchronous release of energy and nitrogen in the rumen is the increased rate of degradation of plant cell wall material in the rumen and a consequential increased feed intake. To test this hypothesis diets in the present study were offered *ad libitum*. The rations for the present study were formulated assuming all the feed was consumed in the first hour of the 24 h period. The work completed by Robinson and McQueen (1994) observed significant differences in the pattern of intakes of animals fed diets varying in their degradation pattern of energy and protein *ad libitum*. Therefore actual patterns of intake were measured during the present experiment so that any differences observed between treatments could be re-related to how they affected the predicted degree of synchrony of the rations.

#### **5.9.1 Milk yield and composition**

Similar to the results found by Herrera-Saldana and Huber (1989) and Aldrich *et al.* (1993) the present trial found a trend towards an increase in milk yield in ewes fed a synchronous diet. Van Saun and Sniffen (1996) concluded that the pre-lactational management of dairy cows significantly affected lactational performance. The ewes in the current experiment were subjected to the same feeding regime and management

practices pre-trial and it is therefore unlikely that results were affected by pre-trial factors. Similarly, other known variants of milk yield and composition cited by DePetters and Cant (1992), namely, environmental temperature, disease, stage of lactation, parity and breed were all similar between treatments and would therefore not be expected to alter the results.

The importance of energy precursor supply during lactation on partitioning of nutrients is highlighted by the work completed by Miettinen and Huhtanen (1996). Increased glucogenic to ketogenic precursors supplied by isoenergetic intra rumen infusions decreased milk fat content but increased milk yield. In the present experiment mean concentrations of volatile fatty acids and relative proportions of individual volatile fatty acids did not alter as a consequence of varying levels of synchronicity. Although propionate and iso-butyrate proportions were higher in animals fed asynchronous diets 3 h after fresh food had been offered, no other differences were observed. It is therefore unlikely that the observed differences in performance can be explained by this small difference in volatile fatty acid proportions.

McCloud *et al.* (1983) indirectly measured the effects of increasing digestible energy supply in isonitrogenous diets (Table 5.13) and found increasing yields of milk and protein with increasing energy to protein ratios, but a decrease in milk fat. Although the alteration in energy supply was manipulated by a change in forage to concentrate ratio and therefore a change in proportions of glucogenic to ketogenic precursors was probable, the same trend in milk production in the present experiment was observed but without a corresponding change in proportions of volatile fatty acids present in the

rumen.

Table 5.13. Effect of increasing energy intake of dairy cows on milk yield and composition.

	Digestible energy intake (DEI,MCal/kgDM)			
	2.83	2.95	3.07	3.19
Crude protein: DEI (g/Mcal)	56.9	54.6	52.1	50.5
Milk yield (kg)	20.8	21.6	22.3	23.4
Protein %	3.11	3.12	3.22	3.26
Lactose %	5.28	5.33	5.33	5.55
Fat %	3.83	3.72	3.68	3.33

Adapted from McCloud *et al.* (1983)

Similar responses to energy supplementation to lactating ruminants have been found by others. Salimei *et al.* (1994) found that Friesian cows fed diets supplemented with bypass fat increased milk yield without affecting milk fat, protein or lactose content indicating a response to increasing the MetE:MetP ratio. Sutton *et al.* (1994) observed that increased energy supply in diets of similar crude protein content significantly increased the efficiency of conversion of dietary nitrogen into milk protein suggesting a sparing effect on dietary protein, possibly from the prevention of protein oxidation to make up energy shortfalls. However this sparing effect has not been recorded by others. For example Hadjipanayioton and Photoin (1995a) and (1995b) found no effects on milk yield or composition from goats or ewes fed diets increasing in protein to energy ratio. However both of these experiments were performed with animals in negative energy balance which

also has an effect on energy metabolism (Ørskov and MacCloud, 1990). Further evidence for protein energy interactions when considering milk composition is supplied by Subnel *et al.* (1994) who estimated that 90% of the variation in efficiency of milk protein synthesis could be explained by the ratio of digestible protein arriving at the intestine to net energy supply. The efficiency decreased when metabolisable protein to digestible energy increased suggesting protein oxidation and/or nitrogen excretion as urea in urine or milk.

Sutton (1989) and Spörndly (1989) both concluded that there is a positive correlation between energy available to the host and milk protein concentration. Spörndly (1989) after analysing 53 feeding experiments calculated that for every 1 MJ increase in metabolisable energy intake a corresponding increase of 0.003-0.005 percentage points in milk protein content could be expected. Although this relationship was measured in dairy cows, the differences observed in protein content in the present study could represent differences in daily metabolisable energy supply.

The rations in the present trial were formulated to supply similar amounts of metabolisable protein per kg dry matter consumed. As average daily dry matter intakes did not vary between treatments it is reasonable to assume that metabolisable protein availability was similar. Aldrich *et al.* (1993) observed significantly higher productions of microbial nitrogen in dairy cows from diets formulated to have similar degradation patterns of energy and protein. Although not measured in the present trial it is possible that synchrony, as found by others (Sinclair *et al.* 1993; Sinclair *et al.* 1995; Newbold and Rust, 1992; Herrera-Saldana *et al.* 1990) increased the bacterial protein supply to the

small intestine due to a more efficient capture of dietary nitrogen. As digestible undegradable protein supply was predicted to be the same for each treatment, an increase in microbial protein would increase the supply of digestible amino acids arriving at the small intestine. However the effects of manipulating milk protein composition by changing the supply of metabolisable protein are highly variable. Some indication that higher protein availability can reduce milk fat have been observed (Sutton, 1989). However Sutton (1989) and Spörndly (1989) concluded that extra protein supplied at the duodenum has little significant effect on milk protein concentration. Aldrich *et al.* (1993), despite observing higher bacterial nitrogen flows due to synchrony, could detect no effect on milk protein content. However, Chilliard and Doreau (1997) found milk protein content to be improved by dietary supplementation with rumen protected methionine. Similarly, Metcalf *et al.* (1996), using infusion techniques, found that intra vascular supply of the essential amino acids associated with milk protein increased the milk protein content of cows milk by 13.5% ( $p < 0.05$ ). It is possible therefore, that increased supply of protein from higher microbial production supply cannot be capitalised upon in terms of milk protein content due to inappropriate amino acid profiles, and the only extra benefit from increased supply of protein is a sparing effect on energy. Other recent work (Pulina *et al.*, 1995; Hof and Tamminga, 1994) has suggested that effects of protein intakes on milk protein content exist but that efficiencies of incorporation may be low.

Positive effects on milk yield and composition have been found through other nutritionally orientated criteria. MacCloud *et al.* (1983) found that dry matter intake was linearly and positively correlated to milk yield. However, Broster *et al.* (1985) observed that increased dry matter intakes of rations of the same concentrate to forage ratio



significantly decreased milk fat content. Average daily dry matter intakes in the present study did not significantly differ and cannot therefore be used to explain the differences in production.

The fatty acid composition of the diet may affect milk fat concentration and milk fatty acid composition (Sutton, 1989). Inclusion of lipid in the diet up to 6-8% of the dry matter content generally increases milk yield although responses to milk fat content are far more variable. The higher inclusions of malt distillers dark grains, fishmeal and tallow in the diets increasing in their degree of asynchrony resulted in higher contents of ether extract (Table 5.3). The overall percentage of fat per kg organic matter consumed was within the range described by Sutton (1989) and therefore could have been expected to boost milk yield. In contrast, the current experiment found animals fed the asynchronous rations (containing higher fat content per kg) had lower milk yields and higher milk fat content. The depressed hydrolytic activities of cellulolytic rumen bacteria sometimes associated with lipid intake (McDonald *et al.* 1988) are generally at higher inclusion levels than the levels used in the current experiment. Evidence that rumen function was not altered to different extents by the various diets is further provided by the similar dry matter intakes and rumen VFA concentrations and proportions.

Although the fatty acid composition of the diets was not measured in the present study the inclusion of fish meal and distillers grains in diets of increasing asynchrony would probably have increased the long chain unsaturated fatty acid content of those diets. These types of oils are known to decrease milk fat concentration (Opstvedt, 1984) and recently Chilliard and Doreau (1997) found that lactating Holstein cows supplemented

with fish oil increased their milk output and significantly decreased the milk fat content. Recently, Phipps *et al.* (1998) showed that replacement of rape seed meal and soya with distillers dark grains reduced the milk fat concentration in the milk. This is opposite to the results found in the present experiment and cannot be used to explain the differences in milk composition found here.

Increases in the ether extract content of a ration has been seen to reduce milk protein content (Chilliard and Doreau, 1997). Sutton (1989) estimated this relationship has a reduction of 0.04% units of protein for each 1% increase in dietary ether extract concentration. Although this regression analysis was based on dairy cow information the results of the present experiment are in agreement with this relationship, indicating that ingredient effects could account for some of the variation in milk protein content observed.

A current review (Reynolds *et al.* 1997) has suggested that starch that bypasses the rumen but which is available in the small intestine may increase milk protein content. Hydrolysis and digestion of bypass starch yields glucose. The requirement for glucose by the small intestine is relatively high and any extra supply as a result of bypass starch may have a sparing effect on glucogenic amino acids, allowing more protein available for milk protein synthesis (de Visser, 1993).

Reynolds *et al.* (1997) state that the response in milk protein content often seen with increased supply of dietary starch may be due to both increased by-pass starch and increased production and concentration of ruminal propionate. In the current trial

concentrations and amounts of propionate were not different between treatments, despite a tendency for milk protein content to increase due to synchrony. However, there were predicted differences in starch content from the three rations.

### **5.9.2 Blood and rumen metabolites**

Blood urea concentrations have been shown to fluctuate in accordance with rumen ammonia concentrations (Holder *et al.* 1995). The significantly lower plasma urea concentrations found in the ewes fed synchronous diets may therefore be caused by lower rumen ammonia concentrations. All diets were formulated to have sufficient levels of rumen degradable nitrogen to support microbial growth on a daily basis (AFRC, 1992). The increased plasma urea observed in diets increasing in their degree of asynchrony may be due to periods of excess ammonia concentration in the rumen. The associated energy costs of nitrogen recycling have been discussed previously.

Elevated plasma  $\beta$ -hydroxy butyrate concentrations were considered by Ørskov and MacCloud (1990) as an indication of a deficiency of glucose pre-cursors. However, the increased concentration of plasma  $\beta$ -hydroxy butyrate may also be accompanied by elevated plasma urea levels caused by oxidation of protein (Ørskov and MacCloud, 1990). Although plasma  $\beta$ -hydroxy butyrate concentrations tended to be higher in animals fed diets increasing in their degree of synchronicity in the present study, the significantly lower plasma urea in animals fed the synchronous diets does not lend support to this theory.

Production of butyrate in the rumen directly influences plasma  $\beta$ -hydroxy butyrate concentrations (MAFF, 1984). However, there was no significant difference in rumen butyrate concentrations in the current experiment. An alternative explanation for the increased levels of plasma  $\beta$ -hydroxy butyrate observed for animals fed the synchronous rations is that noted by Newbold (1994). Animals supplied with excess metabolisable protein may try to obtain extra energy via mobilisation of body reserves. This would tend to suggest that animals fed synchronous diets were mobilising more energy than those fed asynchronous diets.

### **5.9.3 Pattern of intake**

A reason for measuring the pattern of intake in the present experiment was to observe if animals offered a complete and asynchronous diet did alter their pattern of intake to increase the ration's degree of synchrony. Robinson and McQueen (1994) had noted that a more even supply of protein and energy to the rumen of dairy cows led them to consume greater amounts of the ration immediately after fresh food had been offered. Table 5.14 compares the predicted supply of nutrients to the rumen for the current experimental diets as formulated originally (the entire ration consumed in the first hour) and for the pattern of intake measured for the three treatments (Fig 5.3a). All calculations were based on an assumed rumen outflow rate of 0.06 per h. Table 5.14 suggests that the pattern of intakes displayed by the Friesland ewes altered the predicted synchrony indices, although the predicted ranking remained the same.

Pattern of intake differed most markedly in the first hour after feeding as observed by

Robinson and McQueen (1994). Although not significant, animals offered a synchronous supply of energy and nitrogen to the rumen tended to eat a greater proportion of their total intake immediately after fresh food had been offered. This difference in intake had the effect of increasing the initial peak of nitrogen release in synchronous fed animals relative to others, bringing the synchrony indices closer (Table 22).

Table 5.14. Comparison of nutrient supply for predicted and actual patterns of intake displayed by Friesland ewes fed diets differing in their degree of synchrony.

	Diets		
	Syn	Int	Asy
Organic matter degradability			
Predicted <sup>1</sup>	0.58	0.58	0.57
Actual <sup>1</sup>	0.59	0.58	0.58
Nitrogen degradability			
Predicted	0.69	0.72	0.74
Actual	0.69	0.72	0.74
Daily ratio (gN/kgOMTDR)			
Predicted	35	38	41
Actual	35	38	40
Synchrony Index			
Predicted	0.88	0.82	0.61
Actual	0.79	0.74	0.68

<sup>1</sup>Predicted based on all food consumed in the first hour

<sup>1</sup>Actual based on the observed patterns of intake

Forbes (1988) suggested that animals offered food *ad libitum* will consume the greatest proportion of their daily intake in the first hour and reduce their voluntary intake to virtually zero a few hours pre-feeding. All ewes in the current experiment displayed this behaviour. The numerous controls thought to be associated with voluntary food intake

were presented in Table 4.20. The concentrate portion of the diets was ground to a similar size, had similar dry matter content and made up the same proportion of the total ration for each treatment. It is unlikely therefore that differences in patterns of intake were due to differences in rumen distension caused by the concentrate fraction. Asynchrony promoted higher plasma urea concentrations possibly reflecting greater concentrations of rumen ammonia. This increase in ammonia level after feeding may have increased rumen fluid osmotic pressure, a signal to the animal to reduce intake (Carter and Grovum, 1990). High concentrations of rumen ammonia can lead to urea poisoning if excessive (Chalupa *et al.* 1979) and therefore in a response to self preservation plasma or rumen ammonia levels may alter meal size.

Table 4.20 also indicated that rumen volatile fatty acid production and/or concentration may act as chemical controllers of feed intake (Forbes, 1988). Although proportions of individual short chain fatty acids did not vary from zero to one hour post feeding in the present experiment, total volatile fatty acid concentrations were significantly less at feeding in ewes offered synchronous diets (Table 5.9). This may have allowed the greater feed intake associated with these animals immediately after fresh food had been offered.

## 5.10 CONCLUSIONS

A synchronous hourly supply of energy and nitrogen to the rumen tended to increase milk protein content of milk and the daily production of milk protein. However, part of this effect may have been due to differences in ether extract content of the rations offered.

A synchronous hourly supply of energy and nitrogen to the rumen significantly reduced milk fat content.

There were indications of differences in energy metabolism between lactating sheep fed diets differing in rumen synchrony.

Animals offered asynchronous rations *ad libitum* could not change their pattern of intake sufficiently to provide a more synchronous supply of nutrients to the rumen.

## CHAPTER 6

### 6.0 GENERAL DISCUSSION

Ultimately experiments completed by agricultural scientists should aim to improve the efficiency and cost effectiveness of agricultural production. However, for real progress to be made within this concept there also needs to be a synergism of ideas throughout the industry. For the scientists part, this means that results and conclusions from one piece of work may inspire others to take it further and incorporate new aspects. Therefore, there is the need for a broad spectrum of experiments to take place, ranging from more theoretical type studies to farm based trials. The present thesis has attempted to take a theoretical concept, that a more complementary supply of hourly energy and nitrogen to rumen microbes will increase the efficiency with which they grow, and assess this in terms of animal production.

The UK farming system is dynamic and for farmers to stay profitable they must be able to adapt to the changing environment around them. For them to be able to do this they must be given the tools they require. The recent disbandment of the Milk Marketing Board has led to many pricing schemes available for liquid milk based on milk volume, fat and/or protein content. The work completed in the present study indicated that ingredients chosen to be complementary for their hourly release of energy and nitrogen in the rumen significantly altered the content of milk fat in liquid milk. Aldrich *et al.* (1993) found similar results when dairy cows were offered synchronous rations. Results such as these depict the potential for farmers to be able to produce milk according to their



individual milk contracts.

Similarly, the production of both beef and lamb is, financially, becoming less and less viable. Although much of this is due to the strength of the green pound at present, production costs will continue to squeeze margins. The results found in the current thesis suggest that a choice of ingredients that are complementary for their hourly release of energy and nitrogen in the rumen has the potential to increase the efficiency with which lambs grow. Supposing that ration costs were similar, this indicates that the lambs fed the synchronous rations were finished at a lower cost than lambs fed the asynchronous rations.

Throughout the experiments described in the present work there was indications that differences in energy metabolism may occur between sheep offered rations differing in the degree of synchrony. In the growing lamb trials improvements in feed conversion efficiencies and growth rates due to synchrony were observed, as were higher deposits of internal fat reserves and increased rumen weight. Similarly, during the lactation trial elevated plasma BHB levels noted in these ewes maybe an indication of body lipid mobilisation due to periods of dietary energy shortages. In chapter 1 it was noted that the actual  $Y_{ATP}$  values measured in anaerobically grown microorganisms were somewhat lower than the theoretical values produced by Hespell and Bryant (1979, see section 1.3.2). Providing a more complementary supply of energy and nitrogen to the rumen microorganisms may improve the  $Y_{ATP}$  value associated with the microorganisms populating the rumen of that animal. Possible mechanisms for this include the reduction of energy spilling reactions, changes in bacterial cell composition and energy needed for

recycling of N (see Table 1.8). Ultimately, improving the energy efficiency of the rumen and the whole body increases the amount of substrates available to the host for productive purposes. In addition to changes in energy metabolism that may have occurred in the rumen in the present experiment, other speculative areas include the uncoupling of metabolism associated with the production of NADPH<sup>+</sup> from iso-citrate due to a shortage of glucose and/or glucose pre-cursors, and the detoxification of plasma ammonia by the liver associated with high levels of uncaptured rumen degradable protein. The current work provides evidence to suggest that a choice of dietary ingredients that provide complementary rates and extents of energy and N degradation in the rumen, affect the overall energy metabolism of the animal to such an extent that productive capability is affected.

Chamberlain *et al.* (1993) supplemented grass silage based diets with different types of carbohydrates and measured the efficiency of microbial production (Table 1.11). The authors found that although all supplementary sugars and starch improved microbial efficiency over unsupplemented rations, some, particularly sucrose, improved it to a greater extent. This highlights an important area of controversy associated with the synchrony concept. How do you know that it is the synchronous release of nutrients in the rumen and not the individual ingredient that is released causing the effect? The type of work completed by Newbold and Rust (1992) showed that using the same "ingredients" offered at different times to produce different patterns of nutrient release in rumen fluid, resulted in a stimulatory effect on microbial efficiency and production. The ingredients used for the rations in the present work were chosen solely for their degradation characteristics. As a result the asynchronous rations in both the growing and

the lactation experiments contained higher proportions of what are considered as higher quality feeds (e.g. fishmeal), often associated with production responses. These responses were not observed in the present experiments. Similarly, much evidence is available showing that the types of oils supplied in feeds such as fish products and distillers feeds will cause a lowering of milk fat content. An opposite effect was observed in the current experiment. These findings lend support that an improved microbial efficiency, as observed by Newbold and Rust (1992) has the potential to alter animal performance. Animal production experiments that have compared the effects of certain ingredients on animal performance often conclude that one ingredient is better than another. For example the work spoken of above carried out by Chamberlain *et al.* (1993) found sucrose to be a better supplement for grass silage based diets than other sugars. However, is it that the rumen microorganisms preferred this sugar *per se* or is it that this sugar happened to have a rate of solubilisation that was a closer match to the release of nitrogen from the grass silage used ?

Perhaps a more precise methodological approach to assessing the effects of synchrony on animal production is that used by Henderson *et al.* (1998). However, feeding the same ingredients at different times of the day has the potential to cause large differences in rumen conditions. This in itself will cause differences in rumen metabolism. An objective of the current work was to try and incorporate the enhancement of microbial production and efficiency observed by others (Sinclair *et al.* 1993; Sinclair *et al.* 1995; Newbold and Rust, 1992; Herrera-Saldana *et al.* 1990) when fed synchronous rations into practices that could be achieved by farmers. Incorporating a system such as the SIRE computer program (Sinclair, 1992) into current formulation packages would be relatively straight

forward. Its reliance on readily available analysis techniques gives further scope to its commercial applicability.

The experiments completed in the present study have indicated that animal responses to diets formulated to be synchronous for their hourly release of energy and nitrogen in the rumen exist. However, further work is required to find a more precise reason as to why those responses exist. Many more nutritional type studies remain to be completed, therefore, on the effects of synchronising the hourly release of energy and nitrogen in the rumen on host animal performance.

## **7.0 GENERAL CONCLUSIONS**

1. Different batches of the same ingredients may display marked differences in degradability characteristics.
2. The use of ingredients that were complementary for their rates and extents of degradation in the rumen improved the feed conversion efficiency of growing lambs.
3. Synchronous diets fed to Friesland ewes decreased their milk fat content.
4. There are indications that differences in energy metabolism may exist between animals fed synchronous and asynchronous diets.
5. Animals fed asynchronous rations could not alter their intakes sufficiently to provide a more synchronous release of nutrients in the rumen.

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## Appendix 1

ANOVA table.

Diets	2
Sheep	23
Periods	2
<u>Residual</u>	<u>44</u>
Total	71

## Appendix 2

ANOVA table.

Diets	2
Sheep	5*
Periods	2
<u>Residual</u>	<u>8</u>
Total	17

\*2 ewes per treatment (per period)

## Appendix 3

ANOVA table.

Diets	2
Sheep	11*
Periods	2
<u>Residual</u>	<u>20</u>
Total	35

\*4 ewes per treatment (per period)